



BIOCHEMICAL RESPONSES OF SOME PLANTATION CROPS TO AMBIENT AIR POLLUTION

ABSTRACT

Ph. D. THESIS IN BOTANY

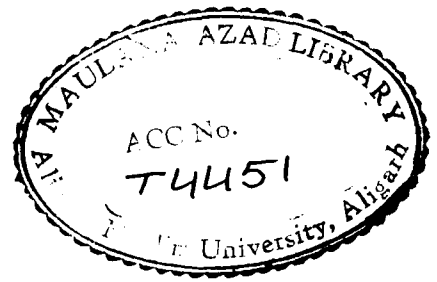


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1993



A B S T R A C T

Sulphur dioxide, one of the by-products of coal burning and a potent phytotoxic pollutant causes severe damage to vegetation. Earlier studies on sulphur dioxide pollution were based on appearance of visible injury symptoms. But the recent studies have brought to light that SO₂ pollution might also affect the plant growth and yield without any visible injury, involving the normal physiological and biochemical processes in plants. Further, much of the information available at hand is based on the studies carried out under artificial fumigation and not on plants growing under natural field conditions. Since trees have a long standing experience of exposure to the ambient environment pollution load than seasonal crops, the present work has been carried out to study the impact of coal smoke pollution on the biochemical responses of some important tree species viz. Dalbergia sissoo, Eucalyptus citriodora, Mangifera indica, Psidium guajava, Syzygium cumini and Tectona grandis. The study includes the estimation of the seasonal variations in the mineral contents (viz. sulphur, nitrogen, phosphorus, potassium), chlorophyll pigments, carotenoids as well as carbohydrate and protein level in the foliage, newly formed bark and in the sap wood samples.

Analysis of Plant Samples :-

The estimation of S, N, P, carbohydrate and protein has been accomplished by following the methods of Patterson (1958), Lindner (1944), Fiske and Subarow (1925), Dubois et al. (1956) and Lowry et al. (1951) respectively. K has been digested by following the procedure given by Lindner (1944) and estimated flame photometrically. Chlorophyll and carotenoid contents have been extracted from the leaf tissue by following the method of Arnon (1949) and their amounts calculated by the formulae given by Duxbury and Yentsch (1956) and MacLachlan and Zalik (1963) respectively. The results obtained are briefly summarized below.

Sulphur Accumulation :-

The sulphate sulphur contents analysed in the foliage of different seasons shows highly significant accumulation in the population growing at the polluted site compared to control. The data also indicates that P. guajava among the fruit trees and D. sissoo among the timber trees posses the highest potential for the accumulation of sulphur in the SO₂ enriched atmosphere. The foliage of various seasons have shown its minimum sulphur concentration in the summer season (regardless of the site of collection), whereas, the maximum has been observed in the monsoon season except in the samples of two deciduous species D. sissoo and I. grandis which exhibit the highest concentration in ✓

their foliage of late winter season collected from the polluted site.

Bark samples collected in various seasons also exhibit highly significant sulphate accumulation in all the species growing at the test site. The highest percent increase compared to control has been recorded in P. guajava followed by S. cumini, E. citriodora, M. indica, I. grandis and D. sissoo. However, no critical variations in sulphur contents have been observed in the wood samples of most of the species except D. sissoo and I. grandis which show a considerable increment in the polluted atmosphere.

Nitrogen Assimilation:-

The data on nitrogen assimilation reveals that the trend of N accumulation is different in the various investigated species. S. cumini is the only species to record the elevated levels of N in its foliage under pollution stress irrespective of any seasonal effect. Whereas, the other species show both losses as well as gains in N level in their foliage compared to control, the magnitude of which being variable depending on the time of collection as well as the inherent characters of the species concerned. Interestingly, in all the fruit trees viz. M. indica, P. guajava and S. cumini, a significant increase in the N accumulation is observed in the late winter season compared to control. Further, the concentration of N in the foliage of M.

indica, P. qualava and I. grandis show the highest amount in monsoon, while S. cumini and D. sissoo have recorded maximum concentration in early winter season, regardless of the site of collection. Unlike others, E. citriodora exhibits the peak concentration in its 15 days old summer foliage.

Bark samples of D. sissoo show reduced N contents in the population growing at the test site. Whereas, the other species though exhibit higher N values in their polluted samples, the percent increase is only significant in the summer samples of M. indica and S. cumini compared to control. Wood samples on the other hand, observe losses in N contents in most of the species growing at the polluted site except S. cumini and I. grandis which record higher values. Moreover, the concentration of N in the wood is higher compared to bark in all the investigated species.

Phosphorus Assimilation:-

The P contents in the foliage of various tree species shows that S. cumini responds negatively under pollution stress in all the seasons. Whereas, E. citriodora and D. sissoo record significantly higher as well as lower P concentrations in their polluted samples compared to control. The other three species viz. M. indica, P. qualava and I. grandis do not show any critical variations in the P contents in either set of the samples, except in the early winter samples of I. grandis where

pollution causes a significant loss. Moreover, the population of both the sites (polluted and non-polluted) yields the maximum concentration of P in the monsoon foliage.

The data on the P contents in the bark samples of various tree species reveals that M. indica and S. cumini show higher values in P contents, whereas, the other species have recorded low levels of P in the SO₂ enriched atmosphere. On the other hand, pollution causes an increase of P contents in the wood samples of M. indica, S. cumini and I. grandis, whereas, the remaining species show losses in the same environmental set-up. However, the concentration of P recorded has been found to be higher in the wood samples compared to bark in all the investigated species.

Potassium Balance:-

The data obtained on the K contents in the foliage of different tree species under study shows a universal depletion in the population growing in the SO₂ enriched atmosphere except I. grandis which observes an increase in K level in its foliage as well as in bark and wood samples. K contents are reduced in the bark and wood samples of the remaining species with the single exception of D. sissoo which records higher K levels in its bark samples under pollution stress.

The concentration of K in the foliage in various seasons is maximum in the summer in case of M. indica,

P. guajava, S. cumini and D. sissoo, whereas, the other two species E. citriodora and I. grandis record the peak concentration in their monsoon foliage regardless of the site of collection.

Chlorophylls and Carotenoids:-

The impact of coal smoke pollution is also considerable on the photosynthetic pigments. The observations show that there is a decrease in the chlorophyll pigments in all the investigated species. However, the maximum loss has been found in chlorophyll a than in chlorophyll b. Out of the six species studied, two species S. cumini and D. sissoo suffer significantly high degrees of losses in chlorophyll contents in all the seasons. Carotenoids also experience reductions due to pollution stress in all the species, the magnitude of loss is generally higher in early winter season.

Carbohydrate Metabolism:-

The carbohydrate contents in the foliage of various seasons in different species show gain as well as losses in the population growing under pollution stress. Among the various species, the highest percent increase has been found in early winter samples of P. guajava and the maximum percent reduction in the summer foliage of S. cumini, both collected from the population growing at the test site. The concentration of carbohydrate in

the foliage of various seasons records lesser values in summer in all the investigated species.

The bark samples of M. indica and P. guajava show significantly higher amounts of carbohydrate in the polluted population except in the monsoon samples of the latter where the difference compared to control is non-significant. On the other hand, summer and monsoon samples of E. citriodora record severe losses in their carbohydrate contents in the SO₂ enriched atmosphere. The other three species viz. S. cumini, D. sissoo and I. grandis do not show any marked variations compared to their controls. The wood samples of all the species growing in the polluted atmosphere invariably exhibit elevated levels of carbohydrates in all the seasons. Further, the concentration of carbohydrate has been found the maximum in the wood samples compared to bark or foliage except in M. indica, in which the foliage shows the highest concentration followed by wood and bark.

Protein Synthesis:-

The protein contents exhibit varied trends in different experimental trees. The population growing under pollution stress show varying amounts of protein in their foliage and the degree of loss or gain depending on the season of sampling as well as the genetic constitution of the species concerned. Compared to other species, the population of M. indica show the

highest degree of increase in protein contents in the late winter season under pollution stress and similarly, the maximum percent reduction has been also observed in the monsoon samples of the same species collected from the same site. The observations also show that protein content increase to a significant level in the late winter samples of all the fruit trees (M. indica, P. guajava, S. cumini) growing in SO₂ enriched atmosphere, whereas, other species do not show any critical variations in their protein contents in the same season.

The bark samples of all the tree species show increased protein contents in the population growing at the test site compared to control except D. sissoo which records a decline. On the other hand, wood samples also experience a decrease in their protein contents in the SO₂ enriched atmosphere in 4 out of the 6 investigated species, viz. M. indica, P. guajava, E. citriodora and D. sissoo. Whereas, the remaining two species S. cumini and I. grandis observe elevated levels of proteins in their polluted samples. However, the amount of protein has been found higher in the wood samples than in bark in all the investigated species.



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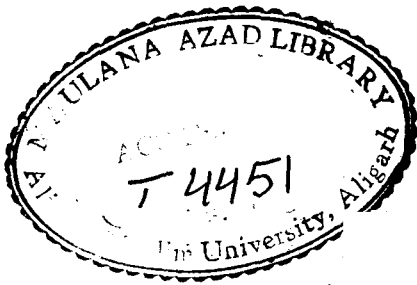
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IN THE NAME OF ALLAH, THE MOST GRACIOUS
THE DISPENSER OF GRACE

- * He (it is who) brings forth the living out of that which is dead, and brings forth the dead out of that which is alive, and gives life to the earth after it had been lifeless.

[Al-Quran, 30:19]

- * Mischief has appeared on land and sea because of (the meed) that the hands of men have earned.

[Al-Quran, 30:41]

- * Nay, but you are people who have wasted their own selves.

[Al-Quran, 36:19]

DEDICATED
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M.Sc., Ph.D., F.L.S., F.A.E.B.

Professor

Dated.....15.12.1993.....

C E R T I F I C A T E

This is to certify that the thesis entitled "Biochemical Responses of some Plantation Crops to Ambient Air Pollution" embodies original and bonafide work carried out under my supervision by Mr. Farooq Ahmad Lone. It may be submitted to the Aligarh Muslim University, Aligarh, towards the fulfilment of requirements for the degree of Doctor of Philosophy in Botany.

A handwritten signature in dark ink, appearing to read 'Samon', with a horizontal line extending to the right.

(A. K. M. Ghouse)

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I bow in reverence to Allah whose gracious blessings gave me the required zeal for the completion of this work.

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Farooq A. Lone
(FAROOQ AHMAD LONE)

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APPENDIX

1. INTRODUCTION

Newton's third law "Every action has a direct, opposite and equal reaction", may as a metaphor, apply equally to man's relationship with nature, as it relates in the formal scientific sense to application of force on inanimate objects. An alternative characterization is that while man sought domination over nature in 5000 years of recorded history, in the last 50 years he has begun to realize that his welfare indeed his very existence, is deeply intertwined with the integrity of natural cycles and systems.

Man is unique in many ways and one of these is his ability to devise new ways of using natural resources for his well being. So long as the requirements of his economic activities were small in relation to global stocks of critical natural resources, he could count through intelligence, cooperation and labour, on improving his welfare. However, his economic activities have increased at an exponential rate in the past several decades and now his requirements have begun to press on the physical limits of nature. The result is the prospect of serious alterations in the earth's resource base and life support systems. The principal manifestations of these impacts are on global climate, the intricate web of forests,

ecology and diversity of living being, and increased transparency of the earth's atmospheric protective shield to harmful ultraviolet radiations. All these impacts are related, directly or indirectly, with man's economic activities and with each other. They all have serious implications for his future well-being.

The deteriorating quality of the environment is causing world wide concern and mankind is faced with newer and unimaginable kinds of environmental problems. Developmental activities all over the world have altered the environmental quality at micro, macro and global levels. Development is accompanied by some form of pollution which threatens not only animal and plant life but the very existence of the human race.

Pollution travelled with man and his civilization from the time of cave dwelling scattered families to the time of urban skyscrapers and metropolis communities. It has grown manifolds with the population and industrial outbreak, and now has reached every nook and cranny of the earth. The increasing tilt towards urbanization and industrial concentration has led, among other things to congestion in residential areas and heavier use of city highways. Pollution increases not only because as people multiply the space available to each person become smaller, but also the demands per person are continually increasing, so that each throws away large quantities of harmful substances year by year. As the earth becomes more crowded,

there is no longer an "away". One person's trash basket is another's living space.

The organic fuel burnt at thermal power stations contains harmful impurities which are injected into the environment as gaseous and solid components of combustion products and adversely affect the atmosphere, water and the whole biosphere. The main pollutants emanating out of coal burning from the thermal power plants are sulphur dioxide, nitrogen oxides, carbon oxides, hydrocarbons, fluorides, fly ash and other particulate pollutants. These thermal power plants use thousands of kilograms of low grade coal per day for power generation. In India by the turn of this century about 70,000 MW of thermal power will be generated using low grade coal. It has also been reported that after the consumption of 80 million tonnes of coal, 1.35 million tonnes of SO_2 is let into the environment (Kumar and Prakash, 1977). According to 1980 estimates, 13 million tonnes of fly ash, 4,80,000 tonnes of SO_2 , 2,80,000 tonnes of NO_x , 16,000 tonnes of CO and 5,000 tonnes of hydrocarbons are released in the atmosphere each year by our thermal power stations (Sharma, 1986). During the past 25 years, we have added quite a few thermal power stations/super thermal power stations and many more are likely to be commissioned during the coming years and thus further aggravating the already serious environmental pollution problem in certain pockets of the country.

Sulphur dioxide is the major phytotoxic pollutant in coal-smoke which may cause serious short and long term effects on vegetation (Whitby, 1939; Thomas, 1951; Pelz, 1956; Stern 1968; Jacobson and Hill, 1970; Van Haut and Stratmann, 1970, Naegel, 1973; Dugger, 1974; Mudd and Kozlowski, 1975; Ziegler, 1975; Mansfield, 1976; Farrar et al., 1977; Hallgren, 1978; Black and Black, 1979; Malhotra and Blanel, 1980; YU and wang, 1981; Ayazloo et al., 1982; Dubey et al., 1982; Last, 1982; Irving and Miller, 1984; Koziol and Whatley, 1984; Malhotra and Khan, 1984; Treshow, 1984; Farooq et al., 1985; Heggestad et al., 1986; Jones et al., 1987; Marchwinska and Kucharski, 1987; Farooq et al., 1988; Fangmeier, 1989; Murray and Wilson, 1990; Sharma and Prakash, 1991; Polle et al., 1992).

In view of the wide spread damage caused to vegetation by air pollution, several centers in India have initiated studies on the phytotoxicity of different pollutants during the past two decades. Ahmad and his colleagues (Ahmad and Yunus, 1985; Yunus et al., 1985; Ahmad et al. 1988) working at National Botanical Research Institute (NBRI) Lucknow, have studied various plant responses in relation to air pollution. Similarly, Rao and his co-workers (Singh and Rao, 1985) at Varanasi; Chaphekar and his associates (Banerji and Chaphekar, 1978; Chaphekar, 1982) at Bombay; Varshney and his group (Varshney and Varshney, 1981) at New Delhi; Dubey and his colleagues (Dubey et al., 1982; Rao and Dubey, 1990a,b) at

Ujjain; Mishra and Shukla (1986) at Kanpur; Das and his co-workers (1981) at Calcutta; Parthasarathy et al. (1975) and Oblisami et al. (1978) at Tamil Nadu and Beg and his associates (Farooq and Beg, 1982; Beg 1988; Beg and Farooq, 1988; Farooq et al., 1988) at Lucknow are the prominent workers who have studied the damaging impacts of several pollutants on the growth and development of plants. A group of researchers belonging to Aligarh Muslim University, Aligarh, have also reported that the effluents coming out from a thermal power plant situated at Kasimpur (Uttar Pradesh) caused damage to some timber trees (Khan, 1982; Ghouse et al., 1984 a,b; Ahmad and Kalimullah, 1986, 1988; Ghouse et al., 1986a; Kalimullah et al., 1987; Gupta et al., 1988; vegetable crops (Amani and Ghouse, 1978; Gupta, 1981; Gupta and Ghouse, 1987a) and various way side weeds (Amani et al., 1979a, b; Ghouse and Khan, 1983, 1984, 1986; Khan, 1985; Ghouse and Saquib, 1985; Iqbal et al., 1986 a,b, 1987, a,b; Mahmooduzzafar et al., 1986, 1987; Saquib, 1989; Usmani, 1990; Malibari et al., 1991; Sabu et al., 1993; Saheed et al., 1993a). Further, anatomical disorders, variations in the leaf epidermal architecture, sulphur catching capacity as well as changes in the amount of photosynthetic pigments of different plant species have been noted under the stress of coal smoke pollution (Ghouse et al., 1980; Khan and Khair, 1984; 1985a, b; Khan et al., 1984; Ghouse et al., 1985; Ghouse and Saquib, 1986; Ghouse et al., 1986b; Gupta and Ghouse, 1986, 1987b; Saquib et al., 1986; Ahmad

et al., 1987; Khan and Ghouse, 1988; Khan and Usmani, 1988; Ghouse et al., 1989; Khan and Khan, 1991; Khan et al., 1991; Saquib and Ahmad, 1991; Lone and Ghouse, 1992; Mahmooduzafar et al., 1992; Ghouse et al., 1993; Lone et al., 1993; Lone and Ghouse, 1993; Saheed et al., 1993b).

The toxicity of SO_2 to plants is manifested in typical chronic or acute foliar symptoms of injury, the extent of which depends upon concentration of noxious gases, fumigation frequency, duration of exposure and operating environmental factors (Thomas and Hendricks, 1956). It has been found that at higher concentrations, plants are reported to exhibit injury symptoms (visible injury) and at lower concentrations, plants are reported to experience certain physiological and biochemical changes (Pierre and Queiroz, 1981; Hallgren, 1984; Heath, 1984; Beg and Farooq, 1988).

A number of environmental scientists have worked out the deleterious impacts of SO_2 on different growth processes of the broad leaved as well as coniferous trees (Katz et al., 1939; Pelz et al., 1963; Linzon, 1965; Prokopiev, 1965; Spierings, 1967; Dochinger, 1968; Dreisinger and McGovern, 1970; Malhotra, 1976; Malhotra and Hocking, 1976; Hallgren, 1978; Malhotra and Sarkar, 1979; Devi and Patel, 1983; Pandey, 1983; Ghouse et al., 1984a, b; Zech et al., 1985; Farooq et al., 1988; Rao and Dubey, 1990a, b; Zech et al., 1990/91). These effects can be quantified by measuring photosynthesis, respiration, leaf conductance,

transpiration, metabolic levels, chlorophyll content, sulphur uptake, biomass and visible injury (Constantinidou et al., 1976; Constantinidou and Kozlowski, 1979; McLaughlin et al., 1979; Winner and Mooney, 1980). Therefore, measuring a physiological response to SO₂ fumigation under field conditions is an important preliminary step in predicting the response of trees to fumigation episodes under field conditions.

Despite the numerous reports on Indian crops and other plant species, the literature pertaining to the impact of coal-smoke pollution on tree species growing in ambient natural conditions is very meagre. Therefore, present work is an attempt in this direction and will elucidate the seasonal variations in the mineral balance, chlorophyll pigments and carotenoids, as well as the status of certain metabolic products like carbohydrates and proteins etc. in the foliage of some economically important tree species. Moreover, the newly formed bark and wood samples of the investigated species have also been analysed for various biochemical parameters which seems to be the unique endeavour of its type at home or elsewhere in the world. The test site for the present study has been selected around a thermal power plant complex situated at Kasimpur township of district Aligarh, Uttar Pradesh.

2. MATERIALS AND METHODS

2.1. GEOGRAPHICAL SET-UP OF THE STUDY AREA

The Aligarh district lies in the North-West of Uttar Pradesh, a Northern state of India, in the fertile agricultural area of Ganga Jamuna Doab between $27^{\circ} 29' N$ and $28^{\circ} 11' N$ latitude and $77^{\circ} 29' E$ and $78^{\circ} 38' E$ longitude (Fig.1). The Kasimpur, the site of Thermal Power Plant Complex, lies in the Morthal Pargana of the Koil Tehsil in the Aligarh district. On the northern border of the town, flows the Upper Gangetic Canal supplying water to the power plant. This place is about 16 km. (road distance) in the north-east of Aligarh city (Fig.2) situated between $27^{\circ} 59' N$ and $28^{\circ} 3' N$ latitude and $78^{\circ} 8' E$ and $78^{\circ} 93' E$ longitude, about 187 meters above the sea level.

2.2. METEOROLOGY

The study area experiences a dry and tropical monsoon type of climate with seasonal rhythm marked by the north-east to south-west monsoon. The year comprises three principal seasons viz. cold weather season (winter), hot weather season (summer) and rainy season (monsoon).

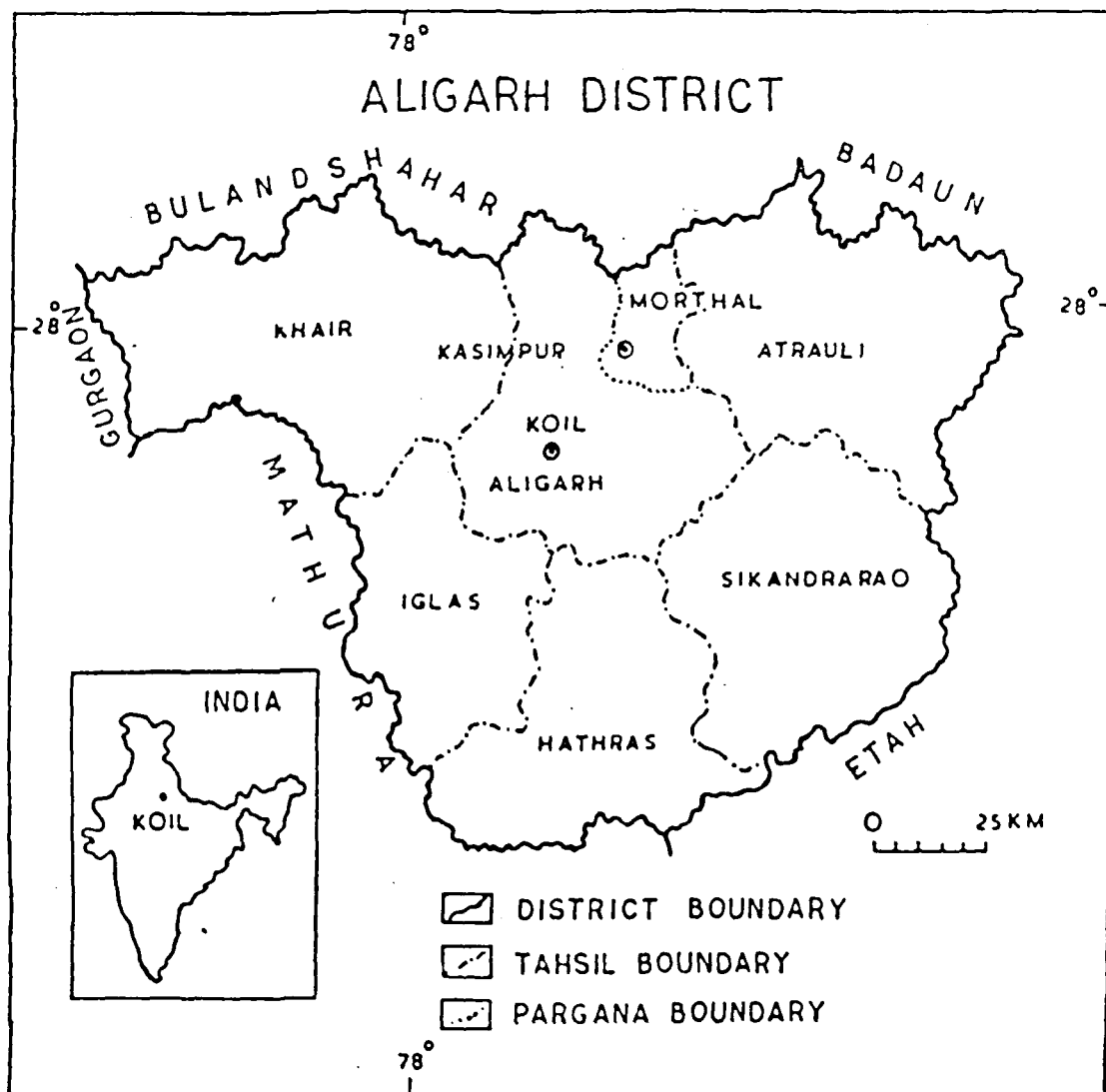


FIG. 1

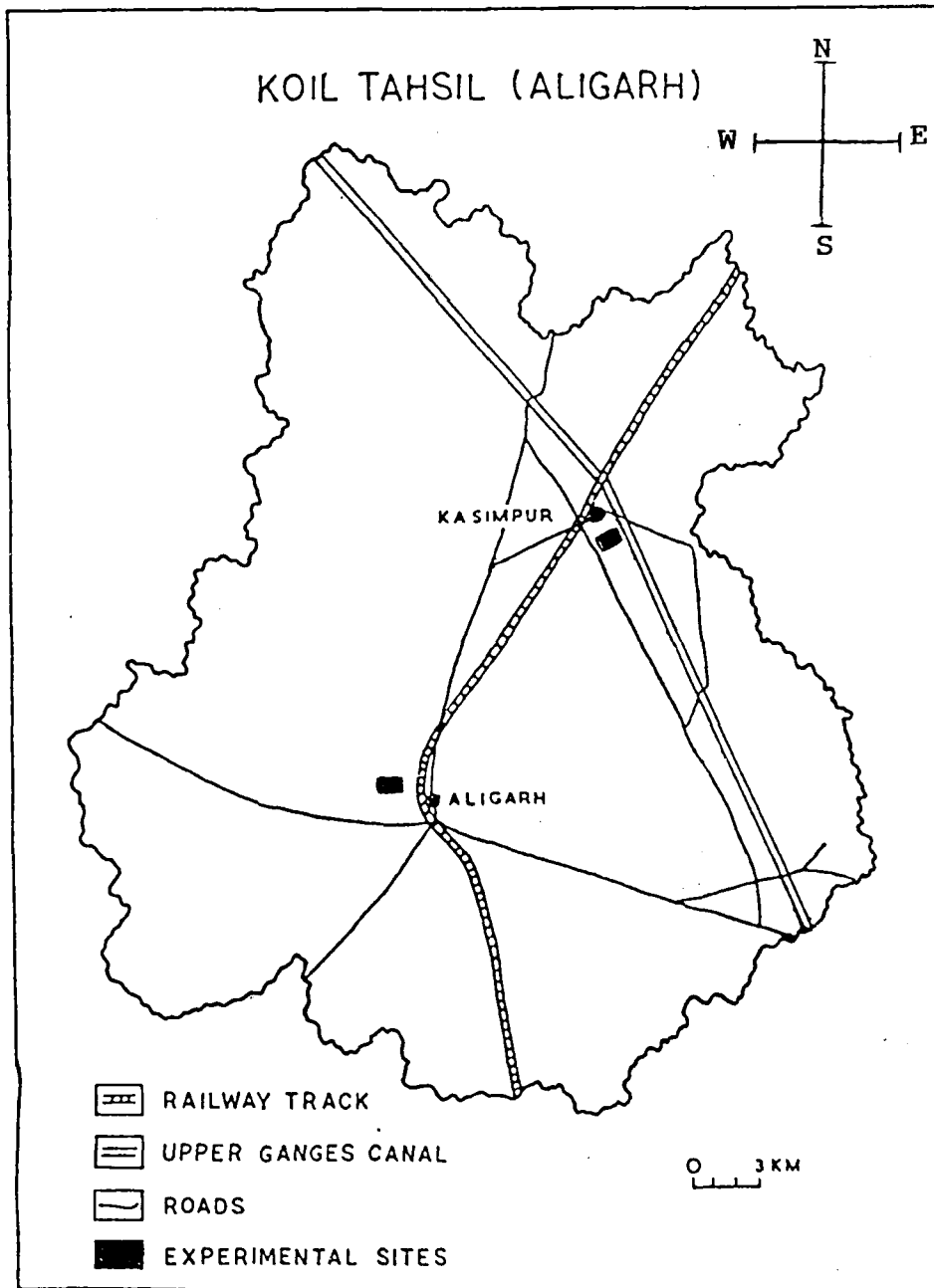


FIG. 2

2.2.1. The Cold Weather Season/Winter (November to March):-

The beginning of winter season is marked by a considerable fall in temperature. In this season, a relatively low pressure exists over the Indian seas, thus causing the winds to blow from plains towards the seas. The mean maximum temperature is 27.11°C in November and 30.14°C in March, while the average minimum temperature for these months is 12.98°C and 14.63°C respectively (Fig.3). It is very cold in the month of January (7.87°C - 21.32°C), the temperature begins to rise (14.63°C - 30.14°C) in March. In winter months the nights are very cold and the days are comparatively warmer with foggy mornings.

Wind direction during the winter season is predominantly from east to west, west to east or south east to north east. The winds during this season are light and blow at an average speed of 3.06 km/hour (Fig.6A). The average relative humidity during this season is 49%, 57.5%, 61%, 52% and 38.5% for months November to March respectively (Fig.5). The rainfall in this season is irregular and sporadic (Fig.4).

2.2.2. The Hot Weather Season/Summer (April to June):-

This season extends from March to June. It begins with an appreciable rise in temperature and a decrease in pressure. Due to wide range of temperature during the summer months, days are warm and nights are pleasant. The minimum and maximum

temperatures in April are 19.01°C and 37.01°C respectively. The temperature continues to rise during May (23.29°C – 39.50°C) and June (26.60°C – 40.86°C) (Fig.3). Days are hot and dry, the average relative humidity declining to 30%, 35% and 44.5% in the months of April, May and June, respectively (Fig.5).

The hot day winds blowing with high velocity form a regular phenomenon. The velocity of wind begins to increase steadily from April with an average wind speed of 4.31km/hour (Fig.6-B). The wind speed rapidly increases during 8 am to 1 pm causing the wind to blow almost with the force of gale during the next 2-3 hours. It then falls suddenly by 6 pm and a calm usually prevails during the night.

Dust and thunder storms are frequent, at times accompanied by rains. The rains are rare, sporadic, short lived and highly variable in amounts. The average monthly rainfall is about 0.75 mm for April, 14.4 mm for May and 23.60 mm for June respectively (Fig.4).

2.2.3. The Rainy Season/Monsoon (July to October):-

The atmospheric temperature falls with the arrival of the humid oceanic currents and the air becomes cool and pleasing by the end of June. The average minimum and maximum temperatures fall to 24.84°C – 34.48°C in July, 24.79°C – 33.38°C in August, 22.54°C – 33.60°C in September and 18.46°C to 33.47°C in October (Fig. 3). The average relative humidity is 72%, 75%, 70% and 52%

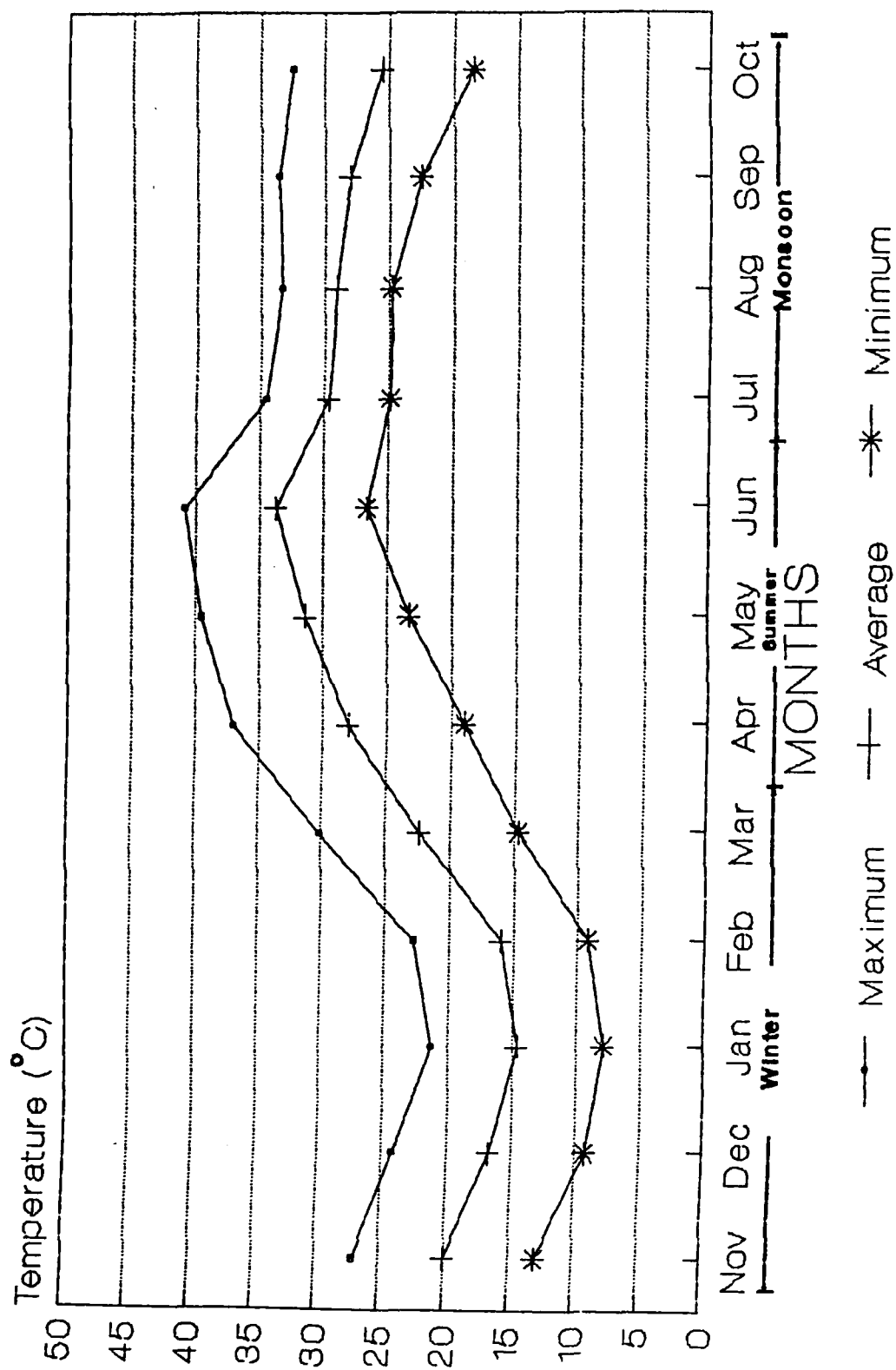


Fig. 3

The graph shows monthly temperature in Centigrade (average of years 1980-1992)

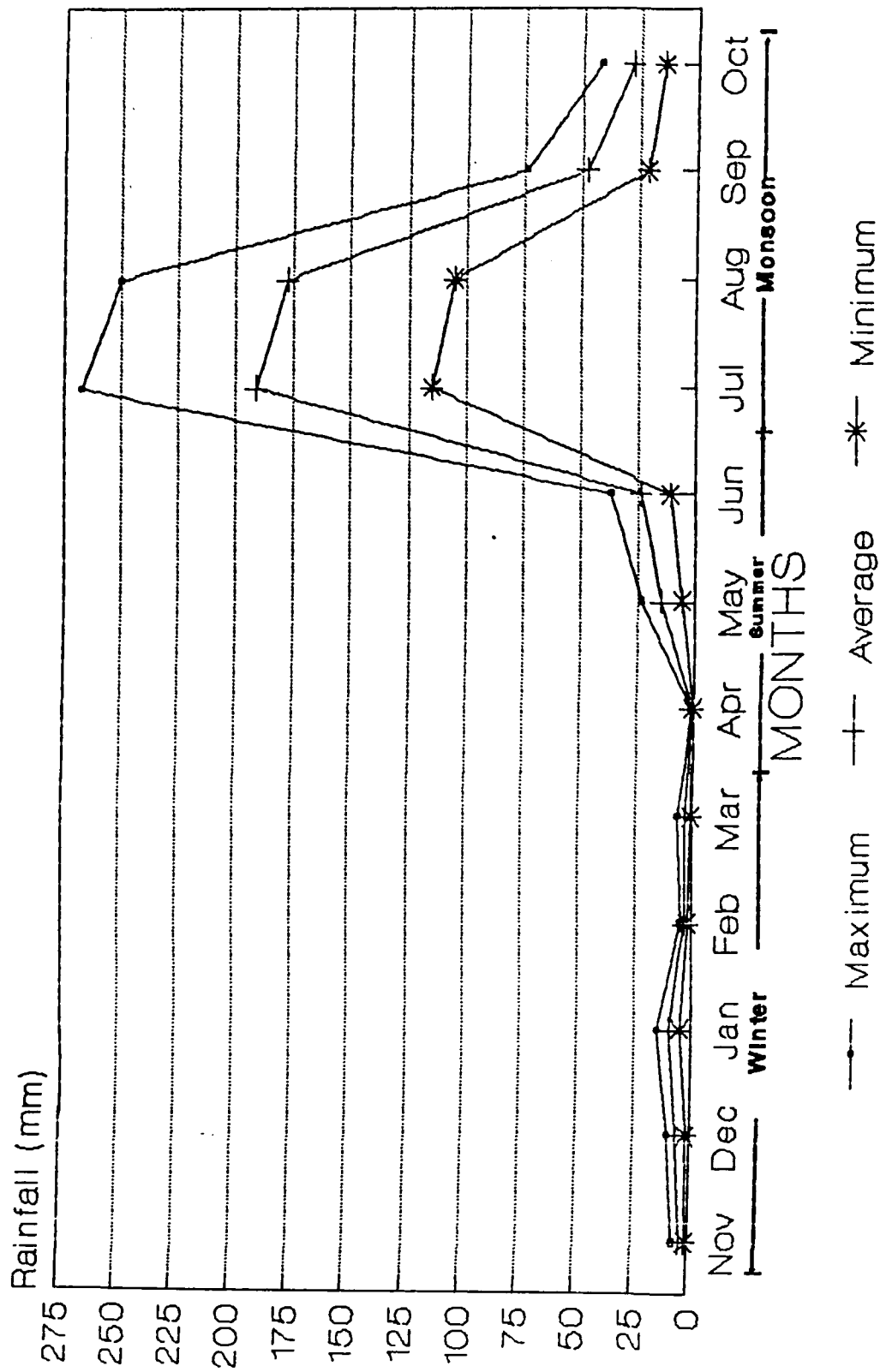


Fig. 4 The graph shows monthly rainfall in mm (average of years 1990-1992)

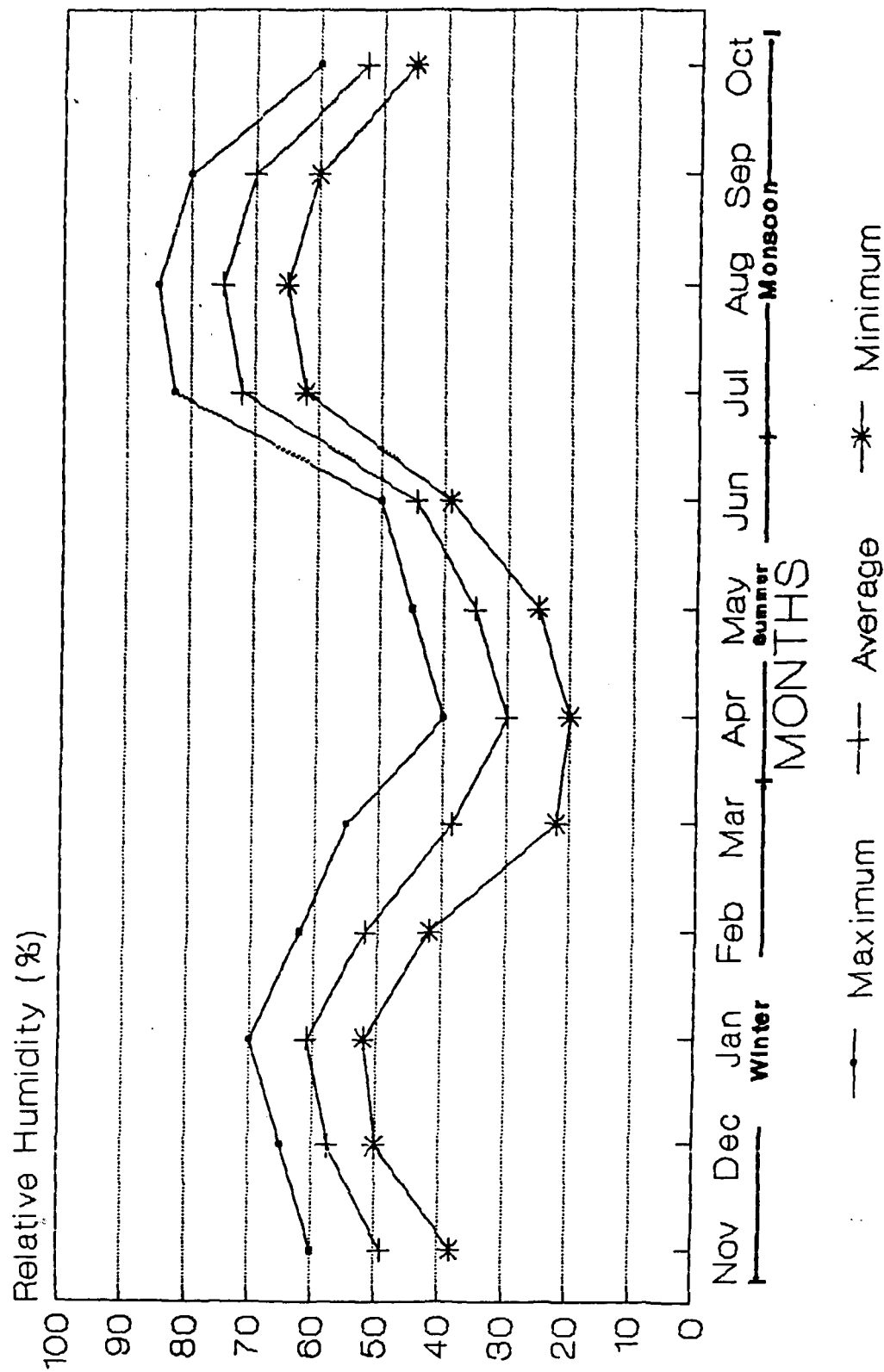


Fig. 5 The graph shows monthly Relative Humidity in percentage (average of years 1990-1992)

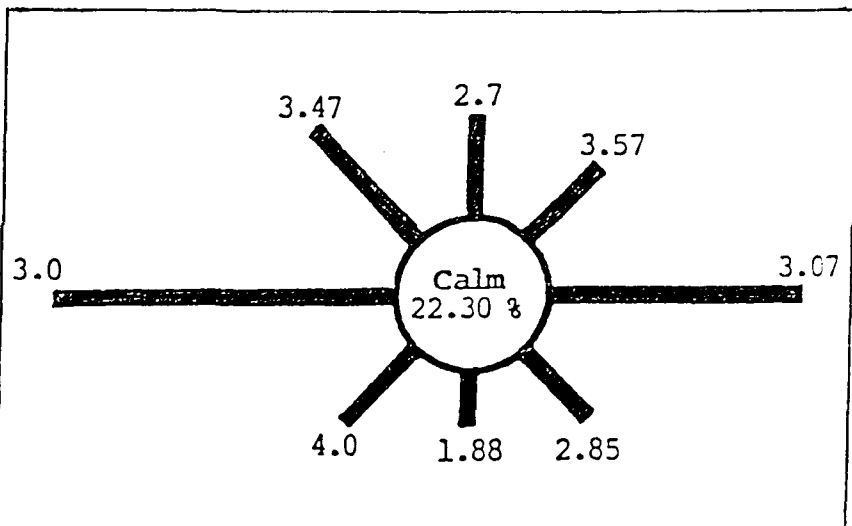


FIG.6A: VELOCITY & PERCENTAGE OF WIND DIRECTION DURING WINTER SEASON.

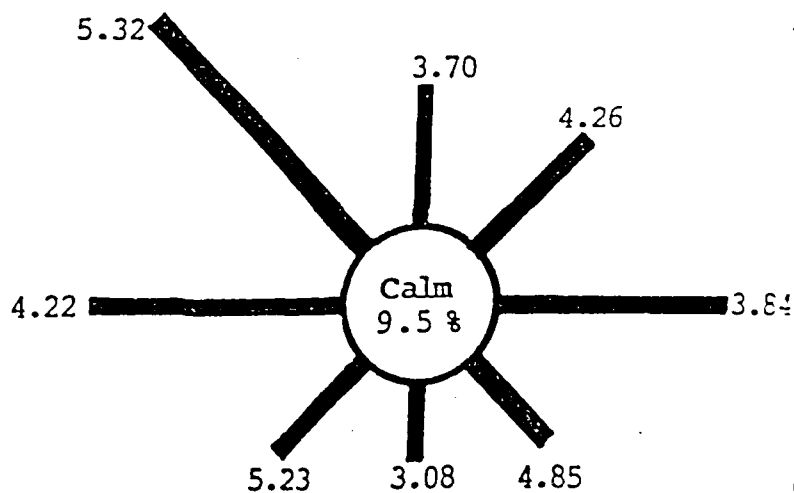
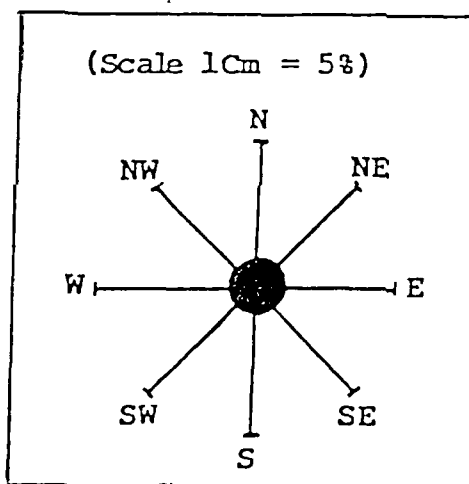


FIG.6B: VELOCITY & PERCENTAGE OF WIND DIRECTION DURING SUMMER SEASON.

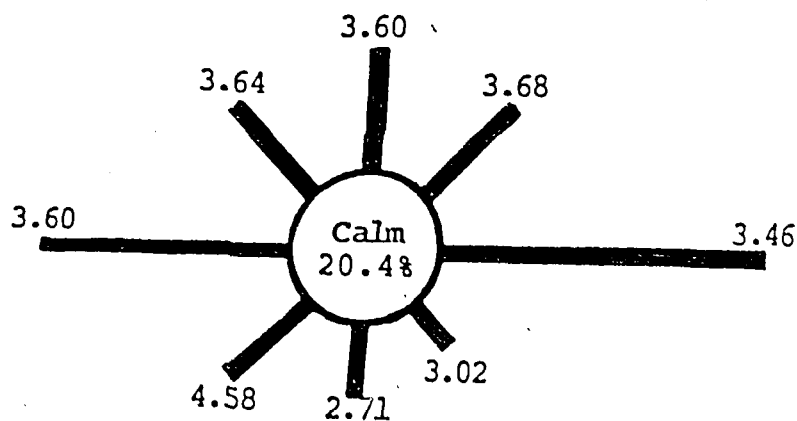


FIG.6C: VELOCITY & PERCENTAGE OF WIND DIRECTION DURING MONSOON SEASON

for these months respectively (Fig.5). The sky is generally overcast. Rain set is usually by the end of June or early July and continues untill the end of September or early October. The average maximum rainfall (about 191mm and 177.5 mm) was observed in the months of July and August respectively (Fig.4). During this season winds blow pre-dominantly from east-west, to west-east, and to south-west to north-east, to south-west and to south-east to north-west (Fig.6C) with an average speed of 3.54 km/hour.

2.3. SOURCE OF POLLUTION

The thermal power plant complex of Kasimpur was found to be the source of pollution. The complex, one of the three major thermal power plants of Uttar Pradesh is located along the banks of the Upper Gangetic Canal running in the north-west to south-east direction and consists of three power stations (A,B and C) having a capacity of 90 MW, 210 MW and 230 MW electricity generation respectively (Plate-I). The whole complex runs on the low grade coal transported from various collieries of North India. The chief chemical constituents of coal are 2.92% moisture, 22.20% ash, 31.96% volatile matter, 0.49% sulphur, 5.61% hydrogen, 5.24% nitrogen, 20.23% oxygen and 42.45% fixed carbon on an average (table-1).

The data (table-2) on coal combustion at the above source shows that annual coal consumption comes to about



Plate - 1

Above : Photograph shows the multi-stack power plant 'A'
 Below : Photograph shows the single stack power plant 'B'
 (left stack) and 'C' right stack

Table-1:- Chemical analysis of coal collected from some important collieries of India.

Name of collieries	percentage								Calorific Value
	Moisture	Ash	Volatile Matters	Fixed Carbon	Sulphur	Hydrogen	Nitrogen	Oxygen	
Badjana	1.00	22.70	31.20	44.20	0.33	5.14	5.10	20.51	5717.10
Bejdih	2.80	22.70	34.00	39.80	0.59	6.99	5.50	20.51	5888.80
Centre Salgram	5.80	19.60	31.20	43.00	0.35	5.21	5.16	20.32	5906.00
Kathara	2.10	20.60	30.60	46.10	0.56	5.14	4.96	19.86	5611.60
Methani	3.00	22.20	32.80	41.40	0.55	5.74	5.46	20.40	5988.40
Poidih	2.80	25.10	31.40	40.20	0.56	5.41	5.23	19.95	5547.70
Average	2.92	22.20	31.86	42.45	0.49	5.61	5.24	20.23	5776.60

Source: Courtesy of AEE, Thermal power plant complex of Kasimpur

Table-2:- Data on coal consumption (in metric tonnes) in the Thermal Power Plant Complex of Kasimpur (An average of data for the years, 1990-1992)

Months	Power Stations			Total Monthly Consumption
	A	B	C	
Nov.	08530	42650	72059	123239
Dec.	09240	53153	83674	146067
Jan.	10992	48070	93579	152641
Feb.	12830	38128	78697	129655
March	18941	56674	85688	161303
April	14338	52810	72844	139992
May	12665	53252	67929	133846
June	08627	38382	58957	105966
July	07920	26623	56011	090554
August	07322	38403	63050	108775
Sept.	08899	42852	66602	118353
Oct.	08240	44974	67110	120324
Total in Winter (Nov. - March)	60533.00	238675.00	413697.00	712905.00
Monthly average	12106.60	47735.00	82739.40	142581.00
Winter daily average	400.88	1580.63	2739.15	1953.16
Total in Summer (April - June)	35630.00	144444.00	199730.00	379804.00
Summer Monthly average	11876.67	48148.00	66576.67	126601.33
Summer daily average	391.54	1587.30	2194.84	4173.67
Total in Monsoon (July - Oct.)	32381.00	152852.00	252773.00	438006.00
Monsoon Monthly average	08095.25	38213.00	63193.25	109501.50
Monsoon daily average	263.26	1242.69	2055.00	3561.00
Annual consumption	128544.00	535971.00	866200.00	1530725.00
Average monthly consumption	10712.33	44664.25	72183.33	127559.58
Average daily consumption	352.17	1468.50	2373.15	4193.74

Source: Courtesy of AEE, Thermal power plant complex of Kasimpur

Table-3:- Amount of gases released from the Thermal Power Plant Complex in different Months (Average of three years, 1990-1992).

Months	Amount of SO ₂		Amount of NO ₂		Amount of CO ₂	
	Kg/hr	ppm/hr	Kg/hr	ppm/hr	Kg/hr	ppm/hr
Nov.	16437	0.016	294416	0.294	7784993	07.785
Dec.	18848	0.019	337696	0.338	11294414	11.294
Jan.	19695	0.020	352894	0.353	14647129	14.647
Feb.	18523	0.019	331869	0.332	14550399	14.550
March	20814	0.021	372920	0.373	17809853	17.810
April	18675	0.019	334590	0.335	1819826	01.820
May	17278	0.017	309442	0.309	2801652	02.802
June	14129	0.014	253152	0.253	2292021	02.300
July	11684	0.012	209354	0.209	1895479	01.900
Aug.	14503	0.015	259862	0.260	2352779	02.353
Sep.	15271	0.015	273623	0.274	2477369	02.477
Oct.	15533	0.016	278180	0.278	2519855	02.520
Average in Winter (Nov. - March)	18863	0.0190	337959	0.338	13217358	13.217
Average in Summer (April - June)	16694	0.0170	299061	0.299	2304500	02.307
Average in Monsoon (July - Oct.)	14248	0.0145	255255	0.255	2311370	02.312
Average	16782	0.0169	300666	0.300	5944409	05.945

Source: Courtesy of AEE, Thermal power plant of Kasimpur

1,28,544 metric tonnes for plant 'A', 5,35,971 metric tonnes for plant 'B' and 8,66,200 metric tonnes for plant 'C'. The total annual coal consumption in all the power plants comes to be about 1,530,715 metric tonnes. The daily average coal consumption is about 4194 metric tonnes. The average monthly and daily coal consumption during the different seasons (winter, summer and monsoon) is given in table (2). On the other hand, the unburnt coal possess considerable amounts of sulphur, nitrogen and carbon and when subjected to 1200°C - 1400°C high temperature for its combustion, obviously produces noxious gases like SO_2 , NO_x and CO_2 . These gases along with other particulate pollutants spread into the atmosphere through various stacks. The computed amounts of SO_2 , NO_2 and CO_2 being released into the atmosphere in kg. per hour and ppm per hour are given in table (3).

2.4. SOIL AND EDAPHIC CONDITIONS

The soil at the test site for the present study has a structure categorised by the Agricultural Directorate (Soil Survey and Research, Aligarh) as type III (Fig.7). This consists of the loam and clayey loam type of soil with a very high pH value and very poor drainage system. During monsoon season it suffers from water logging. The clay content is maximum at the top and decreases with depth. It is ash grey in colour, tending to become black when moist. The poor drainage results in the deposition of soluble sodium salts on the surface in the form of 'Reh'. During

KOIL TAHSIL SOILS

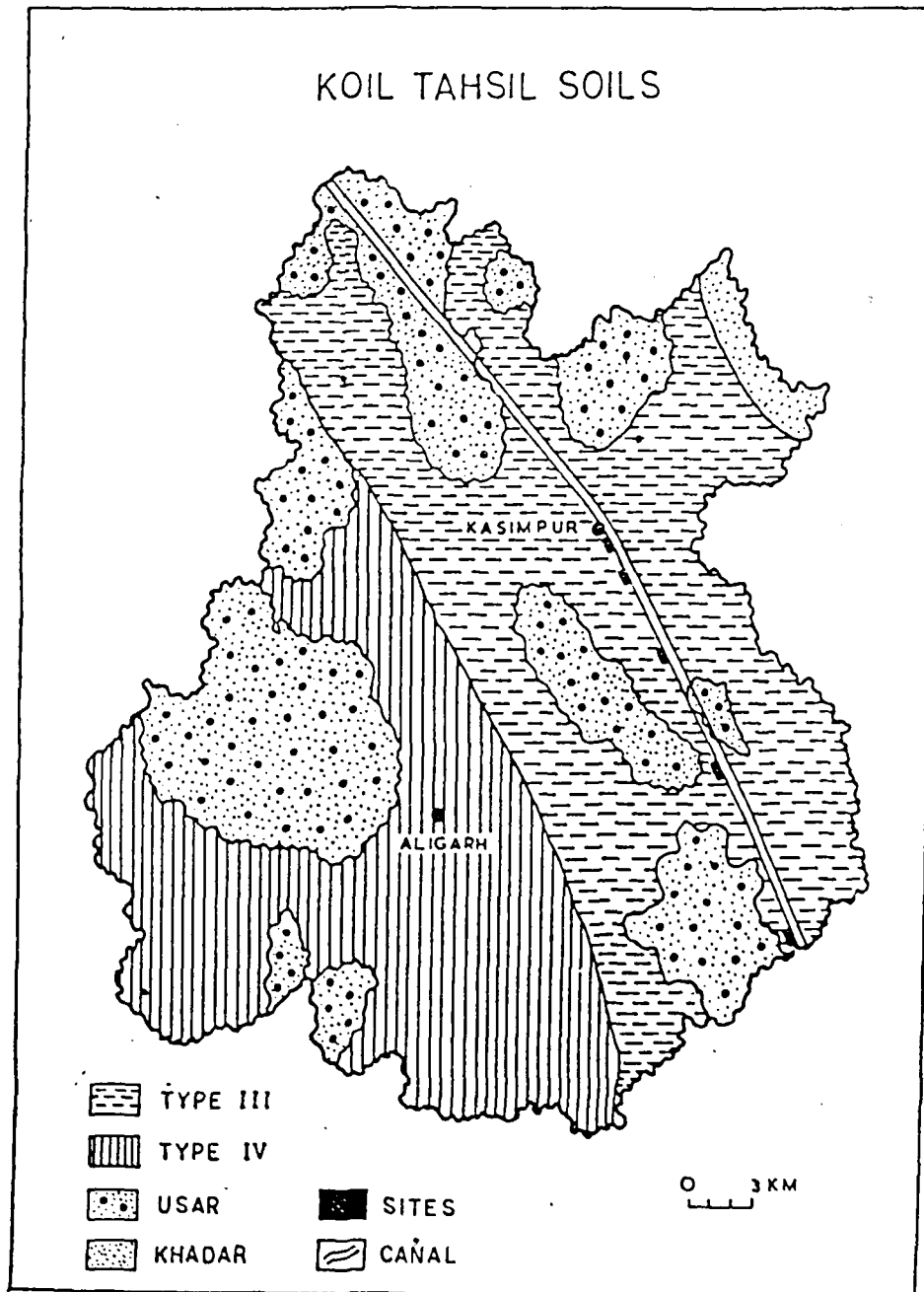


FIG. 7

drought periods, the land becomes white and salt infested. The soil is mostly of alkaline in nature. The data obtained on the mechanical analysis as well as the physico-chemical properties of the soil collected from the test site as well as from the reference site during the study period is given in table (4). It is clear from the data that the sand fraction at both the sites is below 50%. The coarse silt happens to be higher in percentage than the fine silt at both the places. Further, the clay percentage in soil is slightly higher at Aligarh site than at Kasimpur.

The soil pH at both the sites is alkaline, however, the alkalinity is higher at Aligarh site than at Kasimpur. Further, there was a linear increase in soil pH at both the sites from summer to winter seasons. Total nitrogen as well as available phosphorus contents were recorded lesser at the polluted site compared to non-polluted site. On the other hand exchangeable potassium was same in polluted as well as control sites in summer season. However, slightly lesser contents were observed in monsoon and winter seasons at the latter site. Sulphur was found in higher amounts at the polluted site, the maximum contents being observed in monsoon season. Similarly, organic carbon was also recorded higher at the polluted site than at the control.

Table-4:- Mechanical and physico-chemical analysis* of soil samples collected from the polluted and control sites during the study period.

Mechanical Analysis

<u>parameter</u>	<u>Site P</u>	<u>Site C</u>
1. Sand (%) Coarse	1.07	1.87
Fine	48.50	42.21
2. Silt (%) Coarse	16.93	14.90
Fine	10.70	11.82
3. Clay (%)	22.80	29.90
4. Texture Class	Sandy Clay Loam	Sandy Clay Loam

Physico-Chemical Analysis

1. Porosity	46.65	43.24
2. Water Holding Capacity	39.10	35.49
3. E.C. (m.mhos/gm)	4.30×10^{-3}	3.33×10^{-3}
4. C.E.C (m.eq./g soil)	14.30	04.10

		<u>SUMMER</u>		<u>MONSOON</u>		<u>WINTER</u>	
Site-->		P	C	P	C	P	C
5.	pH	7.60	8.00	7.85	8.17	7.87	8.20
6.	Total N(%)	0.070	0.076	0.060	0.063	0.068	0.07
7.	Available P(%)	0.080	0.085	0.110	0.120	0.130	0.14
8.	Exchangeable K(%)	0.077	0.077	0.077	0.075	0.077	0.07
9.	Sulphur (µg/gm)	500	285	525	290	520	290
10.	Organic C (%)	4.10	1.67	5.10	1.90	5.00	1.90

Site P= Polluted Site (Kasimpur)

Site C= Control Site (Aligarh)

* Soil analysis carried out at the Agricultural Directorate (Soil Survey and Research), Aligarh.

2.5. SELECTION OF THE EXPERIMENTAL SITES

Under these ecological and meteorological conditions the test site for the present study was maintained within 1 km. from the pollution source in the down wind direction. The Botanical Garden of the Botany Department of Aligarh Muslim University, Aligarh served as a control site situated at a distance of 16 km. in the cross wind direction from the test site and free of any coal-smoke pollution (Fig-2).

2.6. SELECTION OF SPECIES

After a general survey, the following species of equal age were selected for the present study from both the sites.

- | | |
|-------------------|---------------------------------|
| 1. Botanical Name | = <u>Mangifera indica</u> Linn. |
| English Name | = Mango |
| Family | = Anacardiaceae ✓ |
| Age | = 30 years |

It is a large evergreen tree attaining several meters height. The canopy looks apparently very healthy and is densely covered with dark green foliage. The leaves are alternate, entire and coriaceous. In a calendar year, the growth in this species starts in the month of March in local conditions and the first collection of leaves was made when they were 15 days old. Bark is thick, dark grey, rough with numerous small

fissures and exfoliating scales. Wood is grey to dark brown in colour, coarse grained and soft. This is one of the most important fruit trees of this region.

2. Botanical Name = Psidium guajava Linn.

English Name = Guava

Family = Myrtaceae

Age = 15 years

It is an evergreen small tree upto 5 meters high. Its leaves are light green, pubescent and chartaceous. Fruits are green to light yellow in colour. The first flush of growth starts in the early March and subsequently the first collection was made in the late March. Bark is smooth, thin greenish grey, peeling off in thin flakes. Wood greyish brown, moderately hard and even grained. The fruits are edible and the bark and leaves used for tanning and dyeing. Infusion of leaves is used in cerebral affections and nephritis.

3. Botanical Name = Syzygium cumini Skeel.

English Name = Jambolana, Java plum

Family = Myrtaceae

Age = 25 years

It is a large evergreen tree with much branching and broad canopy and displays a very luxuriant out look. The leaves are soft and shiny. The growth activity starts in the month of March. Bark is light grey, smooth with shallow depressions. Wood is reddish grey, rough and moderately hard. The tree is usually

cultivated throughout India for its edible fruits. A decoction of bark and seeds is said to be useful in diarrhoea and dysentery. Alcoholic extract of seeds has been reported to reduce level of blood sugar in diabetic patients. The decoction of the leaves is reported to be useful in gonorrhoea.

4. Botanical Name = Eucalyptus citriodora Hook.

English Name = Lemon-scented Eucalypt

Family = Myrtaceae

Age = 30 years

It is a tall handsome tree with evergreen habit. Leaves are thick leathery and light green in colour. The first flush of growth starts in late March and the 15 days old leaves were collected in the first fortnight of April. Its bark is grey and exfoliating in long flakes. Wood is hard, reddish brown and close grained. Sap wood is grey and heart wood light brown in colour. It is widely planted as an ornamental tree. An essential oil obtained from leaves and terminal branchlets is used in perfumery. Wood pulp is suitable for preparing writing and printing paper.

5. Botanical Name = Dalbergia sissoo Roxb.

English Name = Sissoo

Family = Papilionaceae

Age = 20 years

It is a large deciduous tree, often planted along road sides and ranks amongst the finest of India's cabinet and

furniture woods. Leaves are compound and leaf-lets smaller in size and dark green in colour. Its bark is thick, grey, exfoliating in narrow longitudinal strips. Wood is very hard and close grained. Sissoo is considered to be the most suitable for making charcoal. Wood pulp is reported to be suitable for writing and printing paper. The first flush of growth starts in the late February and the first collection of leaves was made in the mid March.

6. Botanical Name	= <u>Tectona grandis</u> Linn.
English Name	= Teak
Family	= Verbenaceae
Age	= 25 years

Teak is a large deciduous tree and is the chief export timber of India. Its wood is used for ship building, bridge building, building construction and furniture and cabinet work. Different grades of plywood and black boards are made of teak wood. Unlike others, teak starts its growth activity in the month of May and therefore, the first collection was made in the mid May. Its bark is light brown or grey, fibrous with longitudinal cracks, outer bark peeling off in long thin flakes. Wood is characteristically scented and containing an oil which is easily perceptible to the touch and is preservative. The leaves of teak yield a dye which is used for dyeing wool and silk.

2.7. SAMPLES AND SAMPLING TECHNIQUES

For each species 5 individuals of almost similar age group were selected at each site and from each tree newly emerged 15 days old leaves were sampled in the months of March to May (summer season), depending on the time of initiation of extension growth. The leaves with an apparently healthy look were collected very carefully from all the sides of the tree crown as well as from the top and bottom of the canopy. About 100 samples were collected from each tree, mixed together and thus it served as one replicate. Similarly, the same number of leaves were collected for all individual representatives and the samples collected from 5 trees served as 5 replicates for each species at each site. The sampled leaves were packed in polythene bags and brought to the laboratory in ice boxes. Before being, processed for any chemical analysis the leaves were gently cleaned with moist cotton to remove any particulate matter deposited on their surfaces. Except for chlorophyll and protein estimations where the fresh material was used, the samples were oven dried for other biochemical studies.

The subsequent samplings for similar studies were made in monsoon, early winter and late winter seasons at a respective gap of 120, 240 and 300 days from the date of first sampling of summer season. It was insured that the foliage of last three collections represent the product of first flush and

the foliage developed in the subsequent flushes, if any, was carefully discarded.

Bark and wood samples were also collected from the experimental trees in summer, monsoon and winter seasons, along with the foliage of first three samplings. From each selected individual, two blocks of about 2 cm thick were chiseled out of the main trunk at chest height (1.5 meters from the ground) and each block containing sufficient amount of bark and wood portions. The sampled blocks were processed with stainless steel penknife to separate carefully the portions of wood and new bark. The older and apparently dead parts of the bark were discarded. Thus the sundered wood and bark portions were chopped off in fine slices and sufficiently dried in an oven at 80°C. The bark and wood samples of both the blocks of each replicating individual were mixed and ground in a laboratory grinder to a fine powder and used for desired analysis. The grinder was thoroughly washed and cleaned after every use to avoid contamination.

2.8. PARAMETERS

The samples of leaves, bark and wood of the selected species were analysed on a comparative basis for sulphur, nitrogen, phosphorus and potassium, chlorophyll pigments and carotenoids, as well as carbohydrate and protein contents. The observations recorded on these parameters have been discussed separately

under different heads.

2.9. STATISTICAL ANALYSIS

The data collected on the variations in the amount of different mineral elements and other biochemical parameters in the selected species were statistically analysed as under.

Mean (\bar{X}):-

The arithmetic mean or simple mean or the average value was easily computed by taking the sum of a number of values ($x_1+x_2+x_3+ \dots \dots \dots x_n$), and dividing it by the total number of values (n) involved, thus,

$$\bar{X} = \frac{(X_1+X_2+X_3+ \dots \dots \dots X_n)}{n}$$

$$\bar{X} = \frac{\Sigma X_n}{n}$$

Where $X_1, X_2, X_3, \dots \dots \dots X_n$ are the observations and n is the total number of observations involved.

Standard Deviation (S.D.):-

Standard deviation, or the standard range of observations is the positive square root of the average of sum of the squares of deviations of all observations from their mean.

Symbolically,

S.D. for large samples (δn):-

$$S.D. = \pm \sqrt{\frac{(\bar{X}-X_1)^2 + (\bar{X}-X_2)^2 + (\bar{X}-X_3)^2 + \dots + (\bar{X}-X_n)^2}{n}}$$

S.D. for small samples ($n-1$):-

$$S.D. = \pm \sqrt{\frac{(\bar{X}-X_1)^2 + (\bar{X}-X_2)^2 + (\bar{X}-X_3)^2 + \dots + (\bar{X}-X_n)^2}{n-1}}$$

Where \bar{X} = Mean of the observations involved

$X_1, X_2, X_3 \dots \dots \dots X_n$ = Observations

n = number of observations involved.

Percent Variation (P.V.):-

To show and compare the relative variabilities of two or more sets of measurements entirely in different units, percent variation was calculated. This is the unit less number and measures the magnitude of variation present between the mean of two sites relative to the average of the sites selected as standard for comparison. The formula used for percent variation between different sites is as follows:-

$$\text{Percent Variation} = \frac{X_A - X_B}{X_A} \times 100$$

Where X_A and X_B are the arithmetic means of the parameters at sites A and B respectively.

Student's t-test:-

It was applied to test the significance of the difference between the two sample means (if any), each sample collected from the two study sites.

The following formula was used to compute t-value which was compared with the table value of 't' at their particular degrees of freedom. When the calculated 't' value exceeded the table value the difference between the two samples was treated as significant, otherwise the difference was attributable to a chance factor.

$$t = \frac{\text{Difference of the two sample means}}{\text{Standard error of the difference}}$$

$$\text{or } t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{(S.D._1)^2}{n_1} + \frac{(S.D._2)^2}{n_2}}}$$

Where, \bar{X}_1 = arithmetic mean of one sample

Where, \bar{X}_2 = arithmetic mean of other sample

S.D. ₁ = S.D. of one sample

S.D. ₂ = S.D. of other sample

n_1 = no. of observations of one sample

n_2 = no. of observations of other sample

Degree of Freedom (D.F.):~

Degree of freedom, to be applied to the number of data particularly in t-test was calculated as follows:

$$D.F. = n_1 + n_2 - 2$$

Where, n_1 = no. of observations of one sample

n_2 = no. of observations of other sample

3. COAL SMOKE POLLUTION AND SULPHUR ACCUMULATION

3.1. INTRODUCTION

Sulphur is necessary for the general metabolism of plants as it forms an important component of amino acids, proteins and vitamins. In healthy leaves sulphur content ranges from 500 to 14,000 ppm by dry weight depending upon species (Malhotra and Hocking, 1976). In higher plants sulphur accumulates when applied to the soil in amounts exceeding those optimal for growth (Harward et al., 1962; Saalbach, 1970; Singh and Singh, 1977). The enhanced sulphur content observed under these conditions is predominantly present in sulphate form (Jones, 1962; Jones and Martin, 1964; Jones and Ruckman, 1973; Datko et al., 1978). It has been reported by a number of workers that sulphur can also be absorbed through leaves in the form of sulphur dioxide or sulphur trioxide from the atmosphere. Fisher et al. (1936) reviewed the literature in this regard and reported that the SO_2 of the ambient atmosphere tend to increase the sulphur content of vegetation.

Upon entering through the stomatal opening into the intercellular space of a leaf, SO_2 combines with the water vapours present in the cellular spaces to form sulphurous acid

which in turn form sulphite ions which then get slowly oxidised to sulphate (Thomas *et al.*, 1950). The toxicity of SO_2 may be caused primarily by its oxidation reduction properties rather than by its acidity (Mudd, 1975). The toxicity of sulphite appears to be about 30 times more than that of equivalent sulphate. Thomas and Hendricks (1956) found that sulphate was much less toxic to plants than sulphite. It has been assumed that the oxidation of sulphite to sulphate is an important measure of determining the degree of damage caused by a given exposure. However, Malhotra and Hocking (1976) have found that toxic levels of sulphate are eventually attained leading to injury symptoms.

3.2. METHODOLOGY

For the estimation of sulphate-sulphur, the method given by Patterson (1958) was adopted. The oven dried samples of leaves, bark and wood were ground and passed through 72 mesh screen. 300 mg screened powder and 0.1 ml selenium dioxide (SeO_2) solution was digested using 10 ml conc. HNO_3 and 1 ml of conc. HCl . After filtering the digested material, 10 ml of 3% glycerol was added and volume made upto 100 ml with distilled water. To this solution 5 ml of 2% barium chloride (BaCl_2) solution was added to precipitate sulphur as barium sulphate (BaSO_4). The optical density was noted at 420 nm on a spectrophotometer. The amount of sulphur was determined by freshly prepared standard curve with potassium sulphate solution.

3.3. OBSERVATIONS

Sulphur In Leaves :-

The data summarized in table (5) shows the amount of sulphate contents in the leaves of different seasons collected from the trees growing at the test site as well as from the reference site. In general all the species recorded significantly higher amounts of inorganic sulphur in their foliage in all seasons in SO_2 enriched atmosphere. In all the species investigated in the present study, the concentration of sulphur was recorded minimum in the 15 days old summer leaves of both the sets of plants (polluted and non-polluted) and the maximum in the monsoon foliage except in the polluted samples of two deciduous species viz. D. sissoo and I. grandis which exhibit higher accumulation in their late winter leaves. The observations recorded also indicate that among the various tree species, the lowest concentration of sulphur was recorded in the 15 days old control samples of S. cumini (0.075%) of summer season and the highest in the polluted samples of P. quajava (0.700%) of the monsoon season. Further, it is also clear that, though the concentration of sulphur in the leaves of different species showed altered amounts in various seasons, the pattern of change in the concentration followed a similar trend in polluted as well as control samples (table-5).

The data on the percent variation in sulphate accumulation between control and polluted samples in different seasons showed varied trends in different species. The minimum and maximum percent increase ranged from 24-75% (M. indica), 42-92% (P. guajava), 33-89% (S. cumini), 34-43% (E. citriodora), 36-71% (D. sissoo) and 33-55% (I. grandis). In M. indica, after the appreciably higher accumulation of sulphur by 15 days old leaves (75%) ($P < 0.01$) at the polluted site, there is a gradual decline in the percent variation as the season proceeded till it shows a minimum increase of 24% ($P < 0.05$) in its late winter samples compared to control. Similarly, in P. guajava, after the initial increase in sulphur accumulation upto monsoon (87%) ($P < 0.01$), there is a sharp fall leading to the minimum (42%) ($P < 0.01$) in early winter samples. This was again followed by a rise to show the maximum variation (92%) ($P < 0.01$) in the samples collected in the late winter. The populations of S. cumini and I. grandis showed close resemblance in the trend of sulphur accumulation. In both these species there is a decline from the initial amount, touching the minimum sulphur accumulation in monsoon season (33% in both the cases) where upon it raised to touch the highest (89% in S. cumini and 55% in I. grandis) ($P < 0.01$) in early winter which in turn was again followed by a decline in late winter samples. D. sissoo recorded an altogether different trend. After an initial increase, sulphate accumulation in this species almost remained constant in monsoon and early

Table-5:- Data on the sulphate-sulphur contents (percent dry weight) in the leaves of various tree species in different seasons.

Species	Site	Summer	Monsoon	Early Winter	Late Winter
<u>M. indica</u>	P	0.210±0.020	0.345±0.044	0.265±0.017	0.285±0.030
	C	0.120±0.011 (+75)**	0.250±0.031 (+38)**	0.210±0.026 (+26)**	0.230±0.021 (+24)*
<u>P. guajava</u>	P	0.300±0.025	0.700±0.070	0.355±0.031	0.625±0.035
	C	0.165±0.026 (+82)**	0.375±0.049 (+87)**	0.250±0.034 (+42)**	0.325±0.017 (+92)**
<u>S. cumini</u>	P	0.115±0.020	0.360±0.026	0.265±0.024	0.300±0.034
	C	0.075±0.011 (+53)**	0.270±0.044 (+33)**	0.140±0.014 (+89)**	0.165±0.040 (+82)**
<u>E. citriodora</u>	P	0.175±0.019	0.450±0.029	0.300±0.014	0.340±0.029
	C	0.130±0.023 (+35)**	0.335±0.031 (+34)**	0.210±0.020 (+43)**	0.250±0.018 (+36)**
<u>D. sissoo</u>	P	0.360±0.049	0.505±0.029	0.375±0.050	0.580±0.054
	C	0.265±0.043 (+36)**	0.360±0.073 (+40)**	0.265±0.052 (+41)*	0.340±0.041 (+71)**
<u>I. grandis</u>	P	0.160±0.007	0.325±0.021	0.255±0.037	0.350±0.029
	C	0.110±0.007 (+45)**	0.245±0.034 (+33)**	0.165±0.041 (+55)**	0.230±0.021 (+52)**

Values are mean ± standard deviation.

P = Polluted, C = Control

** = Significant at P(0.01 level

* = Significant at P(0.05 level

(+) indicates percent increase over control

winter (40% and 41% respectively) ($P < 0.01$), although the increase in sulphur accumulation almost doubled in late winter samples (71%) ($P < 0.01$) compared to 15 days old summer leaves (36%). The other species E. citriodora stood alone of its kind in respect of sulphur accumulation. In this species the highest amount of sulphur accumulation in the polluted environment was recorded in the foliage of early winter (43%), while in other seasons, the sulphur content did not differ materially. However, the variation with respect to control was highly significant ($P < 0.01$) at all stages.

Sulphur in Bark :-

The sulphur contents analysed in summer, monsoon and winter seasons (table-6) in the bark samples of the tree species under study shows that the polluted samples exhibited significantly higher levels of sulphur in all seasons compared to control. Among various tree species P. guajava recorded the maximum percent increase in its samples ranging from 56-83% followed by S. cumini (50-60%), E. citriodora (46-55%), M. indica (43-50%), I. grandis (32-47%) and D. sissoo (30-38%). In all the species the maximum percent increase was recorded in the winter samples except in D. sissoo which shows the maximum percent increase in summer season.

The data pertaining to the concentration of sulphate in the samples in various seasons shows that out of six species

Table-6:- Seasonal changes in the sulphate-sulphur contents (percent dry weight) in the bark samples of various tree species.

Species	Site	Summer	Monsoon	Winter
<u>M. indica</u>	P	0.160±0.021	0.165±0.024	0.150±0.010
	C	0.110±0.013 (+45)**	0.115±0.010 (+43)**	0.100±0.014 (+50)**
<u>P. guajava</u>	P	0.125±0.016	0.120±0.013	0.110±0.014
	C	0.080±0.014 (+56)**	0.075±0.013 (+60)**	0.060±0.017 (+83)**
<u>S. cumini</u>	P	0.100±0.017	0.090±0.013	0.080±0.010
	C	0.065±0.012 (+54)**	0.060±0.013 (+50)**	0.050±0.011 (+60)**
<u>E. citriodora</u>	P	0.090±0.018	0.095±0.015	0.085±0.014
	C	0.060±0.013 (+50)*	0.065±0.013 (+46)*	0.055±0.010 (+55)**
<u>D. sissoo</u>	P	0.180±0.014	0.175±0.016	0.160±0.026
	C	0.130±0.015 (+38)**	0.135±0.010 (+30)**	0.120±0.013 (+33)*
<u>I. grandis</u>	P	0.140±0.020	0.145±0.014	0.140±0.017
	C	0.105±0.013 (+33)**	0.110±0.014 (+32)**	0.095±0.011 (+47)**

Values are mean ± standard deviation.

P = Polluted, C = Control

** = Significant at P<0.01 level

* = Significant at P<0.05 level

(+) indicates percent increase over control

studied, three species, M. indica, E. citriodora and I. grandis exhibit the maximum concentration in monsoon season, whereas, the other ones recorded the peak concentrations in summer season (table-6).

Among the various species, sulphate concentration was found lowest in the control samples of S. cumini (0.050%) collected in the winter season, whereas, the maximum in the polluted samples of D. sissoo (0.180%) collected in the summer season. The pattern of change of sulphur concentration in different seasons was found to be similar in both polluted as well as control samples in a particular species. Moreover, the amount of sulphur is much lower in bark as compared to the concentration found in leaves.

Sulphur in Wood :-

The observations (table-7) regarding the concentration of sulphur in the wood samples of various tree species show that most of the species did not exhibit any marked variation between the polluted and control samples, except in D. sissoo and I. grandis where the sulphur content recorded significantly higher values compared to control. The percent increase was about 35% and 37% in summer, 39% and 44% in monsoon and 47% and 50% in winter for D. sissoo and I. grandis respectively. In these two species the concentration of sulphur was recorded the highest in D. sissoo compared to I. grandis. Further, in both the

Table-7:- Seasonal alterations in the sulphate-sulphur contents (percent dry weight) in the wood samples of different tree species.

Species	Site	Summer	Monsoon	Winter
<u>M. indica</u>	P	0.100±0.013	0.110±0.013	0.120±0.015
	C	0.100±0.021 (00)	0.110±0.014 (00)	0.115±0.010 (+4) ^{NS}
<u>P. guajava</u>	P	0.040±0.013	0.050±0.013	0.040±0.011
	C	0.040±0.007 (00)	0.045±0.010 (+11) ^{NS}	0.035±0.007 (+14) ^{NS}
<u>S. cumini</u>	P	0.015±0.007	0.025±0.007	0.015±0.003
	C	0.015±0.003 (00)	0.025±0.008 (00)	0.015±0.007 (00)
<u>E. citriodora</u>	P	0.050±0.014	0.060±0.011	0.055±0.014
	C	0.050±0.010 (00)	0.055±0.014 (+9) ^{NS}	0.055±0.010 (00)
<u>D. sissoo</u>	P	0.115±0.021	0.125±0.024	0.110±0.020
	C	0.085±0.013 (+35) [*]	0.090±0.010 (+39) [*]	0.075±0.007 (+47) ^{**}
<u>I. grandis</u>	P	0.055±0.007	0.065±0.010	0.045±0.007
	C	0.040±0.007 (+37) ^{**}	0.045±0.007 (+44) ^{**}	0.030±0.007 (+50) ^{**}

Values are mean ± standard deviation.

P = Polluted, C = Control

** = Significant at P<0.01 level

* = Significant at P<0.05 level

NS = Non-significant

(+) indicates percent increase over control

species, the concentration of sulphate was higher in monsoon and the lowest in the winter and the variations in the change of concentration followed the same trends in polluted as well as control samples. In general the concentration of sulphur in wood samples was the lowest compared to bark and leaves.

3.4. D I S C U S S I O N

Sulphur in Leaves :-

The increase in the sulphur contents of plants upon SO_2 fumigation is directly related to the sulphate content of the plants (Weigl and Ziegler, 1962). Decormis (1969) found that upto 98% of the labelled sulphur was in the cells in the form of sulphate soon after SO_2 exposure. Faller *et al.* (1970) reported that sulphate is enhanced upto 2300% upon fumigation with sublethal dosages of sulphur dioxide, whereas, organic sulphur increased by a factor of only 2 to 3%. The excess of sulphur in leaves accumulated as sulphate provides a useful measure which can be used as an index to study the level of SO_2 pollution (Pandey, 1983). But Trlica *et al.* (1985) opposed this assumption and are of the view that it is the total sulphur content of the leaf which might provide the useful information rather than the inorganic sulphur alone. However, Legge *et al.* (1988) reported that the ratio of accumulated sulphur (inorganic sulphur) to the assimilated sulphur (organic sulphur) is a useful parameter in

assessing the degree of sulphur pollution. Efflux analysis has revealed that the excess of sulphate taken up by the plant is mainly stored in the vacuoles (Jones, 1962; Thoiron et al., 1981; Cram, 1983) and once excess sulphur has entered a plant's cell, it may be stored directly or after conversion into a storage form, or else it may be translocated out of the cell directly or after conversion into a transportable form. The excess sulphur may be translocated to other parts of the plant and/or released into the environment (Rennenberg, 1984). Further, sulphur level in the plants, however, may be affected by dilution by new growth, movement to other parts, losses through leaching, gaseous emission and exudation by roots (Garsed, 1984).

In the present study the accumulation of sulphate in the leaves collected in various seasons to a highly significant level in the population growing at the test site indicates greater entry of SO_2 into the leaf system in the polluted atmosphere which can affect different physiological and biochemical processes (Rao and Dubey, 1988, 1990a,b). The data (table-5) clearly indicates that out of six species studied, P. guajava among the fruit trees and D. sissoo among the timber trees possess the highest capacity to mobilize and accumulate sulphur under normal conditions. Under the sulphur enriched ambient conditions also the two above referred species showed the highest concentration of sulphate in all the seasons.

Several other workers in the past have also observed increased sulphur contents in the foliage of plants growing in SO₂ enriched atmosphere (Chesselet and Lalou, 1965; Wixson and Jennett, 1975; Guderian, 1977; Pandey and Rao, 1978; Garsed et al., 1979; Lauenroth et al., 1979; Linzon et al., 1979; Furukawa et al., 1980; Mishra, 1980; Elkies and Ormrod, 1981; Garsed et al., 1981; Laurence et al., 1982; Prasad and Rao, 1982; Pandey, 1983; Saxe, 1983; Alekseev and Rak, 1985; Jagger et al., 1985; L'Hirondelle and Addison, 1985; Lockyer, 1985; Murray, 1985; Trlica et al., 1985; Zech et al., 1985; Heggestad et al., 1986; Nyomarkay et al., 1986; Bytnerowicz et al., 1987; Mass et al., 1987; Iqbal, 1988; Legge et al., 1988; Singh and Rao, 1988; Kropff et al., 1989; Amundson et al., 1990; Clarke and Murray, 1990; Murray and Wilson, 1990, 1991; Rao and Dubey, 1990a,b; Schatzle et al., 1990; Sharma and Prakash, 1991; Van der Stegan and Myttenaere, 1991; Zech et al., 1990/91; Dueck and Elderson, 1992; Lone and Ghouse, 1993). Apart from this general aspect of sulphur relationship, the six investigated species exhibited various degrees of sulphate accumulation depending upon its inherent characters without affecting the general pattern.

Among the six species investigated, S. cumini has shown the least amount of sulphur content in the 15 days old leaves (summer season), both under control conditions (0.075%) as well as in the sulphur enriched atmosphere (0.115%). M. indica and I. grandis are the other two species which

showed comparatively poor ability to accumulate sulphur in the early stages (15 days old leaves) under normal conditions, although under polluted conditions they too absorb and accumulate significantly higher amounts of sulphur (table-5). E. citriodora with apparently healthy foliage, however, has shown comparatively higher amount of sulphur concentration over the others namely M. indica, S. cumini and T. grandis.

The higher accumulation of sulphate in the foliage under pollution stress compared to control may probably be due to the following reasons:

- The greater intake of SO_2 from sulphur enriched atmosphere coupled with poor metabolic and growth activities is likely to leave most of the sulphur in an unassimilated form resulting in poor dilution.
- Due to reduced flowering and fruiting in the polluted population, there is a greater possibility of lower translocation of sulphate leading to higher accumulation in leaves.

It becomes quite clear from the data given in table (5) that in M. indica there is a highly significant increase of about 75% in the sulphate contents of 15 days old foliage of the polluted atmosphere compared to control. The developing leaves being physiologically active, it is possible that the enhanced sulphate content may be due to their greater absorption of SO_2 in the ambient atmosphere coupled with the greater translocation

of sulphate from the older twigs. Materna and Kohout (1963) also observed the translocation of ^{35}S from dormant needles of spruce and pine exposed to about 3.4 ppm of $^{35}\text{SO}_2$ for 24 hours. However, other workers (Katz and McCallum, 1939; Maclean et al., 1986) did not find any sulphur accumulation in dormant needles of certain coniferous species and its subsequent translocation to newly growing organs.

The data (table-5) obtained in the present study also shows that in general all the species recorded the minimum concentration of sulphate in their young foliage compared to the older ones collected in other seasons regardless of the level of pollution. This may be due to the fact that sulphate may be metabolized to organic forms of sulphur more quickly in the young leaves than in the older ones (Elkiey and Ormrod, 1981). These findings are also in accordance with the observations of Jager (1976), Guderian (1977), Lauenroth et al. (1979) and Vander Stegan and Myttenaere (1991) who found lesser sulphate concentration in younger leaves as well as in the younger parts of developing leaves, regardless of whether they were exposed to pollution source or not. Moreover, it is also clear that out of six species investigated in the present study, 4 evergreen species (M. indica, P. guajava, S. cumini, E. citriodora), sulphate concentration recorded the maximum in the monsoon samples, which may be due to the active growth period of these trees in this period. Whereas, in the remaining two

species D. sissoo and T. grandis, both deciduous, the highest concentration of sulphate was observed in the foliage of late winter season collected from the polluted site. These observations gain support from the findings of Huttunen et al. (1985) and Pakkala (1986), who recorded the maximum sulphur content in scots pine and Hypogymnia physodes respectively during winter months than in summer. This may be regarded as an adaptive measure of the plants towards SO₂ pollution stress as these two species usually shed their leaves at winter break and serve as carriers for the unwanted inorganic sulphur and thus helping the plant to escape from the excessive sulphur load which otherwise may disturb its various physiological processes (Rao and Dubey, 1990a,b).

Further, it is also possible that the tree species with significantly higher sulphate accumulation in their leaves may get rid of this excess sulphur by translocating it to other parts of the plant. Sulphate is known to be easily translocated in the xylem and phloem (Vander Stegan and Myttenaere, 1991). The phloem transport of the sulphate is evident from the investigations of Biddulph (1956) and Biddulph et al. (1958) and also has been demonstrated in the tobacco and castor bean after foliar application of labelled sulphate (Rennenberg et al., 1979; Bonas et al., 1982). The supporting evidence of sulphate accumulation in the bark samples as observed in the present study (table-6) is the clear indication of sulphate transport

from one part of the plant to other. Bark being a part which breaks off seasonally, the storage of any excess sulphur will easily be excreted from the main stream.

The other possibility of avoiding excess sulphur in higher plants involves the emission of hydrogen sulphide (H_2S) which was first observed by DeCormis (1968, 69). Since then a number of investigations have been published showing that plant cells emit H_2S , when supplied with excess of sulphur dioxide/sulphite (Silvius *et al.*, 1976; Wilson *et al.*, 1978; Winner *et al.*, 1981; Hallgren and Fredriksson, 1982; Taylor and Tingey, 1983; Filner *et al.*, 1984) or sulphate (Spaleny, 1977; Wilson *et al.*, 1978; Winner *et al.*, 1981; Rennenberg *et al.*, 1983) and involves three possible pathways. Since a major part of the sulphur dioxide/sulphite absorbed by leaf tissue is oxidised to sulphate (Sekiya *et al.*, 1982) and generation of H_2S from this sulphur source is a light dependent process, light dependent reduction of sulphate to sulphide may be part of the pathway leading to H_2S emission in response to sulphur dioxide/sulphite; this reduction may be followed by release of sulphide from carrier-bound sulphide. Another possible pathway may be the incorporation of the sulphide moiety of carrier bound sulphide into cysteine and its subsequent degradation to H_2S , ammonium and pyruvate. The third pathway of H_2S generation may be the direct reduction of sulphur dioxide/sulphite to H_2S .

Sulphur in Bark :-

The sulphate contents analysed (table-6) in the new bark samples of species investigated in the present study show significantly higher percent variations compared to control. The presence of sulphate in the bark indicates the translocation from other exposed parts like the foliar organs or the root system. When excess sulphate is taken up by roots, the transportation of the sulphate in the xylem may exceed the need of sulphur in leaves. However, an enhanced transportation of sulphate in the xylem does not necessarily mean that more sulphate is taken up by the leaves, as a surplus of sulphate translocated in an acropetal direction may be retranslocated through the phloem in a basipetal direction (Rennenberg, 1984). Phloem transport of the sulphate is evident from the observations of Biddulph (1956), Biddulph et al. (1958), Rennenberg et al. (1979) and Bonas et al. (1982). Moreover, upon foliar application, [³⁵S] sulphur dioxide is rapidly metabolized in the fed leaf in the light and in the dark (Garsed and Read, 1977a,b), with sulphate as the main product (Garsed and Read, 1977a). When [³⁵S] sulphur dioxide was fed to a single leaf, radioactivity was distributed all over the plant, irrespective of the leaf age (Garsed and Read, 1977a,b; Garsed and Mochrie, 1980). Although sulphite is phloem mobile, sulphate is the major compound translocated upon foliar application of sulphur dioxide. Thus, the high mobility

of radioactivity upon feeding of [^{35}S] sulphur dioxide to a leaf is mainly the result of a translocation of sulphate (Garsed and Read, 1977b; Garsed and Mochrie, 1980).

Thus, in view of the above earlier reports it is clear that sulphate accumulation in the bark samples to a significant level in various tree species growing in the polluted atmosphere investigated in the present study might have either come from leaves (which are exposed to the ambient atmosphere) in a basipetal direction or it might have passed through ray system from xylem into phloem, if the plant absorbs excess sulphate from the soil. The data indicates that the sulphate accumulation was maximum in *P. guajava* showing an increase of about 56%, 60% and 83% in its summer, monsoon and winter samples respectively, compared to control. The next species with high sulphur accumulating capacity is *S. cumini*, followed by *E. citriodora*, *M. indica*, *I. grandis* and *D. sissoo*.

The accumulation of sulphate in barks to a significant level in the trees growing in polluted atmosphere in the present study may be regarded as an adaptive measure to avoid its excess under SO_2 pollution load. Since bark is not the permanent part of the tree and usually gets peeled off after successive intervals, it paves way for the accumulated sulphur to escape from the plant body and avoids any possible set back into its various physiological activities.

Sulphur in Wood :-

The wood samples of various tree species analysed for their sulphur contents (table-7) did not show any significant variations in most of the species except in D. sissoo and I. grandis which exhibited significantly higher accumulation compared to control. The percent increase in the sulphur accumulation ranged from 35-47% in D. sissoo, whereas, in I. grandis the increase was a little higher in the winter samples of the polluted environment showing a 50% increase in sulphate compared to control. A similar increase of about 51.61% in the sulphur contents has been observed in the wood tissues of Pinus echinata Mill. growing in sulphur enriched atmosphere at Cumberland plateau of Kentucky (Ray and Winstead, 1991). Zech et al. (1990/91) also observed significantly increased sulphur contents in the wood samples of declining population of Fagus sylvatica L. growing in a SO₂ polluted area of Ne-Bavarian mountain.

4. COAL SMOKE POLLUTION AND NITROGEN ASSIMILATION

4.1. INTRODUCTION

Nitrogen is one of the essential nutrients for the plants and perhaps nitrogen's most recognised role in plants is its presence in the structure of protein molecules. In addition, nitrogen is found in such important molecules as purines, pyrimidines, porphyrins and coenzymes. Purines and pyrimidines are found in the nucleic acids, RNA and DNA essential for protein synthesis and transfer of genetic material (Devlin and Witham, 1986). The porphyrin structure is found in such metabolically important compounds as the chlorophyll pigments and cytochromes essential in photosynthesis and respiration. Coenzymes are essential to the function of many enzymes. In addition to its absorption generally in the form of NO_3^- , nitrogen is also taken up as NH_4^+ . Once inside the plant, nitrogen is reduced and incorporated into diverse organic compounds (Beevers and Hageman, 1969). Its deficiency may result in stunted growth and yellowing of leaves on account of loss of chlorophyll.

It is an established fact that foliar levels of different inorganic elements are affected by (1) uptake from the

soil and atmosphere (2) translocation to and from other tissues within plant (3) loss of leaching or volatilization (Van den Driessche, 1974; Miller, 1984; Johnson et al., 1985). Further, uptake, accumulation and distribution of various mineral nutrients in the plant body are influenced by several factors such as climate, season, time of the day, age of the tree and foliage, type of the tree species, soil conditions (Bazilevic and Rodin, 1964; Ovington, 1968, Evans, 1979).

4.2. METHODOLOGY

The oven dried samples of leaves, bark and wood were powdered and passed through 72 mesh screen. The samples were then digested by following the method of Lindner (1944).

Digestion of Samples :-

100 mg dry powder of the sample was taken in a 50 ml Kjeldahl flask. Two ml of pure H_2SO_4 (BDH) was added and the mixture was heated for about two hours to dissolve the powder. This acid turned the contents black. After cooling the flask for about 15 minutes, 0.5 ml of chemically pure 30% hydrogen peroxide was added dropwise. The solution was again heated for about 30 minutes, until it turned light yellow in colour and then cooled with 3-4 drops of H_2O_2 , it was reheated for about 15 minutes to get a clean extract. Excess of hydrogen peroxide was avoided

which would otherwise oxidise the ammonia in the absence of organic matter. The peroxide digested material was transferred to 100 ml volumetric flask with three or four washings with double distilled water (DDW) and the volume made upto mark. This served as the stock solution for the estimation of N, P and K.

Estimation of Nitrogen :-

A 10 ml aliquot of the peroxide digested material was transferred to a 50 ml volumetric flask. To this, 2 ml of 2.5N sodium hydroxide was added to neutralize the excess of acid partially. To prevent the turbidity, one ml of 10% sodium silicate was added to the flask and the volume made up. In a 10 ml graduated test tube, 5 ml of aliquot of this solution was taken and added with 0.5 ml of Nessler's reagent (Appendix-1.1) and followed by thorough shaking. The final volume was made up with DDW and kept for about 5 minutes for the maximum colour development. This solution was taken in a colorimetric tube and its optical density measured at 525 nm on a "spectronic-20 colorimeter". A blank was run simultaneously during determination. A standard curve of known dilution of ammonium sulphate solution was made and the reading of each sample compared with the calibration curve. Nitrogen in each sample was calculated in terms of percentage on dry weight basis.

4.3. OBSERVATIONS

Nitrogen in Leaves :-

The data on the contents of Nitrogen (percentage dry weight) in the leaves collected in various seasons from the species investigated in the present study is summarized in table (8). The data reveals that in M. indica, the N contents in the foliage of summer and monsoon seasons, suffered significantly ($P<0.01$) higher losses due to pollution stress, the magnitude of which being a little higher in monsoon (18%) than in summer season (15%). The samples collected in early and late winter, however, showed a gain in the polluted samples, the percent variation over the control being more than three fold in late winter samples (50%) ($P<0.01$). In P. guajava as compared to control, only the summer collections were found to undergo significant ($P<0.01$) reductions in their N contents (19%) while in the leaves of other seasons (monsoon, early and late winter), there was a gradual increase of 7%, 12% and 25% respectively. The N contents in the foliage of S. cumini responded positively with the advancing seasons showing significantly ($P<0.01$) higher amounts of accumulation in the polluted samples compared to control except that of monsoon samples in which the difference proved to be statistically non-significant. The variation was minimum in 15 days old summer leaves (19%) ($P<0.01$) and later it remained almost constant in early and late winter foliage

exhibiting 37% and 35% ($P < 0.01$) variation respectively. In E. citriodora the N contents in the leaves collected in first two seasons recorded higher accumulation in polluted samples compared to that of control. While the increase was only non-significant in the 15 days old summer samples, the monsoon foliage exhibits highly significant ($P < 0.01$) enhancement (29%). However, the leaves of early and late winter months suffered only non-significant losses. In D. sissoo highly significant ($P < 0.01$) increase in N accumulation was observed in summer (26%) and early winter samples (16%), whereas, the monsoon foliage responded negatively experiencing a severe loss of about 17% ($P < 0.01$) in the SO_2 enriched atmosphere. The late winter leaves in this species though showed an increase but the difference on statistical analysis proved to be non-significant. In I. grandis marked ($P < 0.01$) reductions were observed in summer and early winter samples (18% and 19% respectively), whereas, the N accumulation in the foliage of other seasons did not show any critical difference in the polluted environment.

It is also clear from the data (table-8) that in M. indica, P. guajava and I. grandis the N concentration show an initial increase in both the sets of plants (polluted and non-polluted) and the maximum amount was observed in monsoon samples, after which there was a gradual dilution as the season progressed till it touched its minimum in late winter foliage. However, in S. cumini after an initial fall in monsoon leaves

Table-8:- Data on the total nitrogen contents (percent dry weight) in the leaves of various tree species in different seasons.

Species	Site	Summer	Monsoon	Early Winter	Late Winter
<u>M. indica</u>	P	2.05±0.184	3.15±0.212	2.45±0.141	1.35±0.100
	C	2.40±0.107 (-15)**	3.85±0.260 (-18)**	2.30±0.228 (+7) ^{NS}	0.90±0.100 (+50)**
<u>P. guajava</u>	P	2.30±0.141	3.15±0.111	2.70±0.141	1.25±0.070
	C	2.85±0.129 (-19)**	2.95±0.192 (+7) ^{NS}	2.40±0.130 (+12)*	1.00±0.070 (+25)**
<u>S. cumini</u>	P	1.60±0.141	1.05±0.187	2.05±0.130	1.55±0.154
	C	1.35±0.070 (+19)**	1.00±0.088 (+5) ^{NS}	1.50±0.170 (+37)**	1.15±0.130 (+35)**
<u>E. citriodora</u>	P	3.85±0.184	3.35±0.202	2.40±0.141	1.40±0.130
	C	3.75±0.170 (+3) ^{NS}	2.60±0.141 (+29)**	2.50±0.114 (-4) ^{NS}	1.45±0.070 (-3) ^{NS}
<u>D. sissoo</u>	P	2.45±0.158	2.70±0.141	3.95±0.170	2.50±0.070
	C	1.95±0.130 (+26)**	3.25±0.141 (-17)**	3.40±0.100 (+16)**	2.35±0.141 (+6) ^{NS}
<u>I. grandis</u>	P	1.85±0.114	3.65±0.170	3.05±0.171	1.60±0.114
	C	2.25±0.200 (-18)**	3.90±0.141 (-6) ^{NS}	3.75±0.130 (-19)**	1.50±0.184 (+7) ^{NS}

Values are mean ± standard deviation.

P = Polluted, C = Control

** = Significant at P<0.01 level

* = Significant at P<0.05 level

NS = Non-significant

(+) and (-) indicate percent increase and decrease over control respectively

(minimum concentration), the N contents enhanced to touch its peak value in early winter samples which however dropped in the leaves collected in late winter. In D. sissoo there was a continuous increase in N contents as the season proceeded upto early winter, where upon it declined in the foliage of late winter season. E. citriodora behaved differently and recorded the maximum amount of N in its 15 days old summer samples followed by a steady fall with the advancement of season. Among the various tree species, the lowest concentration of N was observed in the late winter foliage of M. indica collected from the reference site (0.90%), whereas, the highest in the early winter samples of D. sissoo (3.95%) collected from the test site. Moreover, the concentration of N though showed alteration in the foliage of different seasons, the change in the concentration followed the same trend in both the sets of samples (polluted and healthy).

Nitrogen in Bark :-

The data (table-9) indicates that the N concentration was significantly ($P < 0.01$) higher in the summer samples of M. indica (30%) and S. cumini (51%) both collected from the polluted site. However, no significant variation was observed in their monsoon and winter samples. On the other hand, D. sissoo experienced heavy losses in its N contents in the summer and monsoon seasons showing a variation of 24% and 21% ($P < 0.01$)

Table-9:- Seasonal changes in the total nitrogen contents (percent dry weight) in the bark samples of different tree species.

Species	Site	Summer	Monsoon	Winter
<u>M. indica</u>	P	0.43±0.033	0.67±0.077	0.44±0.046
	C	0.33±0.030 (+30)**	0.58±0.056 (+16) ^{NS}	0.40±0.068 (+10) ^{NS}
<u>P. guajava</u>	P	0.33±0.042	0.55±0.032	0.94±0.068
	C	0.30±0.020 (+10) ^{NS}	0.50±0.070 (+10) ^{NS}	0.86±0.084 (+9) ^{NS}
<u>S. cumini</u>	P	0.50±0.070	0.75±0.057	0.96±0.079
	C	0.33±0.064 (+51)**	0.70±0.042 (+7) ^{NS}	0.88±0.064 (+9) ^{NS}
<u>E. citriodora</u>	P	0.50±0.070	0.99±0.094	0.80±0.042
	C	0.50±0.031 (00)	0.90±0.106 (+10) ^{NS}	0.74±0.045 (+8) ^{NS}
<u>D. sissoo</u>	P	0.65±0.036	0.70±0.034	0.80±0.073
	C	0.85±0.040 (-24)**	0.89±0.049 (-21)**	0.90±0.068 (-11) ^{NS}
<u>I. grandis</u>	P	0.62±0.045	0.95±0.070	0.75±0.068
	C	0.60±0.026 (+3) ^{NS}	0.88±0.050 (+8) ^{NS}	0.70±0.034 (+7) ^{NS}

Values are mean ± standard deviation.

P = Polluted, C = Control

** = Significant at P<0.01 level

NS = Non-significant

(+) and (-) indicate percent increase and decrease over control respectively

respectively. The other three species viz. P. guajava, E. citriodora and I. grandis although exhibit higher values of N in their bark samples in SO₂ enriched atmosphere, the difference on statistical analysis proved to be non-significant. The bark samples collected in various seasons recorded the maximum concentration of N in monsoon in case of M. indica, E. citriodora and I. grandis whereas, other species like P. guajava, S. cumini and D. sissoo exhibit maximum values in their winter samples. The trend in the change of concentration of N in various seasons showed resemblance between control and polluted samples (table-9). Among all the species, the concentration of N was the lowest in the control samples of P. guajava (0.30%) collected in the summer season and the highest in the monsoon samples of E. citriodora (0.99%) collected from the polluted site.

Nitrogen in Wood :-

The observations recorded on the contents of N in the wood samples collected in different seasons as well as the percent variation between control and polluted samples is shown in table (10). The data reveals that significant ($P < 0.01$) losses are recorded in the polluted samples of M. indica with percent decrease observed maximum in summer season (27%) followed by winter (22%) and the lowest in monsoon (20%). E. citriodora also responded negatively in the polluted environment experiencing a

severe decline of 33% and 21% ($P < 0.01$) in its summer and monsoon samples respectively, whereas, D. sissoo recorded marked reductions only in its winter samples (23%) ($P < 0.01$). In contrast, a highly significant ($P < 0.01$) increase in N concentration was observed in the monsoon and winter samples of S. cumini (33% and 56% respectively). I. grandis also exhibited an elevated level of N ($P < 0.01$) in its summer and winter samples, however, much higher in the former (43%) than in the latter (22%), whereas, its monsoon samples show equal amounts in polluted as well as control sites.

Among different seasons the concentration of N was maximum in the monsoon samples in case of M. indica and E. citriodora whereas, the rest of the species exhibit the peak concentrations in their winter samples. The data also indicates that the lowest concentration of N was observed in the summer samples of P. guajava (0.43%) and the highest (1.66%) in the control samples of M. indica collected in the monsoon season. Moreover, the concentration of N though variable among different species, the change of concentration in a particular species followed a similar pattern in polluted as well as in the control samples (table-10). The data also shows that in general the concentration of N was found higher in the wood than in bark samples in all the species investigated in the present study.

Table-10:- Seasonal alterations in the total nitrogen contents (percent dry weight) in the wood samples of various tree species.

Species	Site	Summer	Monsoon	Winter
<u>M. indica</u>	P	0.44±0.036	1.33±0.048	0.96±0.072
	C	0.60±0.035 (-27)**	1.66±0.108 (-20)**	1.23±0.086 (-22)**
<u>P. guajava</u>	P	0.43±0.048	0.63±0.057	1.08±0.163
	C	0.43±0.063 (00)	0.69±0.054 (-9) ^{NS}	1.22±0.108 (-11) ^{NS}
<u>S. cumini</u>	P	0.67±0.046	1.00±0.077	1.50±0.134
	C	0.60±0.070 (+12) ^{NS}	0.75±0.032 (+33)**	0.96±0.073 (+56)**
<u>E. citriodora</u>	P	0.50±0.059	1.26±0.073	0.81±0.065
	C	0.75±0.040 (-33)**	1.60±0.070 (-21)**	0.82±0.057 (-12) ^{NS}
<u>D. sissoo</u>	P	0.86±0.070	0.88±0.066	1.10±0.080
	C	0.87±0.048 (-1) ^{NS}	1.00±0.104 (-12) ^{NS}	1.43±0.084 (-23)**
<u>I. grandis</u>	P	0.93±0.064	1.00±0.076	1.41±0.070
	C	0.65±0.036 (+43)**	1.00±0.070 (00)	1.16±0.045 (+22)**

Values are mean ± standard deviation.

P = Polluted, C = Control

** = Significant at P<0.01 level

NS = Non-significant

(+) and (-) indicate percent increase and decrease over control respectively

4.4. D I S C U S S I O N

Nitrogen in Leaves :-

Air pollutants are well known to influence mineral accumulation in plants directly or indirectly. The chemical reaction of SO_2 with plants is primarily due to its acidic nature, which in solution, disturbs both the mineral status of the plant and its physiological processes (Thomas, 1961). Udo and Fayemi (1975) observed immobilization of nutrients in oil polluted soil. SO_2 lowers mineral availability to plants in contaminated soil and thus increase their vulnerability to pollution (Tamm, 1976). However, Labeda and Alexander (1978) reported an increase in the **nitrogen** level of NO_2 treated soil due to enhanced biological oxidation. An increase in the level of N in plants exposed to NO_2 due to its foliar absorption was also recorded by Hill (1971).

The data (table-8) obtained on the concentration of N in the foliage of various tree species investigated in the present study shows that the concentration varied in different seasons. As it is clear from the data that out of the six species investigated, three species M. indica, P. guajava and I. grandis record the highest N concentrations (in both the sets of samples) in the monsoon season. However, in S. cumini and D. sissoo the maximum concentration was observed in the leaves of early winter season. E. citriodora on the other hand, behaved in

an entirely different manner recording the maximum concentration in the summer leaves followed by a continuous loss as the season advanced. Several other workers in the past (Malkonen, 1974; Evans, 1979; Garsed et al., 1981) have also observed an increase in N concentration in young leaves which decreased with the increasing age of the leaves. Guha and Mitchell (1966) observed seasonal variations in the N concentrations in the leaves of deciduous trees and showed that the concentration of some elements quantitatively decreases before leaf fall.

It is also clear from the observations that in comparison to control the N accumulation show a highly significant loss in summer and monsoon leaves of M. indica which however observed increased values in early and late winter seasons. Similarly, in the polluted population of I. grandis, the N accumulation exhibits decreased values upto early winter season from summer. The other two species P. guajava and D. sissoo experienced severe losses, however, only in their summer and monsoon samples respectively. The behaviour of S. cumini happened to be unique. Since it is observed that N accumulation increases in its foliage in all the seasons in the SO₂ enriched atmosphere. Whereas, in E. citriodora only the monsoon samples responded positively showing significantly higher N accumulation compared to control. The decrease in the N accumulation in SO₂ fumigated plants has also been earlier reported by several workers in conifer foliage (Malcolm and Garforth, 1977) in

Triticum aestivum (Pandey and Rao, 1978), Mentha piperita L. (De Santo et al., 1979); Arachis hypogea (Mishra, 1980); Petunia spp. (Elkiey and Ormrod, 1981); Pinus sylvestris (Garsed et al., 1981); Cicer arietinum, Oryza sativa, Panicum miliaceum; Vicia faba (Agrawal, 1982); Phaseolus aureus (Sharma and Rao, 1985) and Hordeum vulgare (Prakash et al., 1989a). Even under the ambient field conditions around a thermal power plant, certain broad leaved tree species like Diospyros melanoxylon, Lagerstroemia parviflora and Zizyphus nummularia have shown significantly reduced N contents in their foliage (Pandey, 1983). Zech et al. (1990/91) have also observed decreased values for N contents in declining Fagus sylvatica populations growing in an SO₂ polluted area of Ne-Bavarian mountain.

The loss in the N contents in the polluted population may be due to the loss in protein synthesis (Pahlich, 1973) or due to the inactivation of enzymes responsible for protein synthesis (Cecil and Wake, 1962). Further, it may be possible that the nutrient supplying capacity of the polluted soil (table-4) particularly with respect to N might be less as reported earlier by Agrawal et al. (1985).

Inspite of the loss in N accumulation at certain growth stages in the population growing in the polluted atmosphere, the increased amounts have also been recorded in the present study. The retarded growth activity of the plants in the polluted atmosphere coupled with lesser metabolic activities

might have left N in the plant body unutilized and thus gets accumulated. Further, it is also possible that plants may absorb and fix NO_2 from the atmosphere and thus increase their N contents. Since NO_2 is also one of the by-products of coal burning and therefore its presence in the ambient atmosphere at the test site is inevitable. It is an established fact that plant leaves can absorb many kinds of gaseous air pollutants, including NO_2 , through their stomata (Spedding, 1969; Rich et al., 1970; Porter et al., 1972; Okano et al., 1989). Lower concentrations of NO_2 absorbed by the leaves can be easily metabolized and detoxified if relevant enzymes are active (Rogers et al., 1979; Yoneyama and Sasakawa, 1979; Rowland et al., 1987). After foliar entry gaseous NO_2 reacts with extracellular water forming nitrite and nitrate ions which are incorporated in the N metabolism of the cell by the action of nitrite and nitrate reductases. The nutritional effect of NO_2 on the plants in N deficient conditions has also been reported by several workers in the past (Troiano and Leone, 1977; Troiano, 1978; Yoneyama et al., 1980; Srivastava and Ormrod, 1984; Okano and Totsuka, 1986; Rowland et al., 1987; Wingsle et al., 1987; Sabaratnam et al., 1988). These facts lead us to an idea that vegetation could be exploited for reducing the concentration of NO_2 in the ambient atmosphere. The increase in N contents in plants has also been observed after fumigating them with NH_3 (Draaijers et al., 1989; Bobbink et al., 1990; Dueck and

Elderson, 1992) or after exposing them with acid mist containing major S or N pollutants (Kimball et al., 1988; Jacobson et al., 1989).

It is also clear from the observations (table-8) that the three fruit trees (M. indica, P. guajava, S. cumini) show significantly higher N accumulation compared to control in their late winter leaves, which of course seems to be an adaptation developed by the plant so as to translocate most of it towards the newly emerging leaves which may need higher N supply during stressed conditions. Sacher (1957) and Das (1968) have also reported that minerals are transferred to green or living parts when the leaves began to dry.

Nitrogen in Bark :-

The data regarding the concentration of N in the newly formed bark samples of the tree species undertaken for investigation in the present study is summarized in table (9). The data reveals that in M. indica and S. cumini significantly higher N accumulation was observed in their summer samples of the polluted environment as compared to control. Whereas, it was significantly reduced in the summer and monsoon samples of D. sissoo collected from the same site. Other species like P. guajava, E. citriodora and I. grandis did not show any significant variation in their N contents compared to control. Further, the concentration of N was the highest in the monsoon

samples of M. indica, E. citriodora and I. grandis whereas, the other three species viz. P. guajava, S. cumini and D. sissoo show the peak concentrations in their winter samples. Moreover, all the species exhibited similar trends in the change of N concentrations in their polluted and control samples

Nitrogen in Wood :-

The observations (table-10) based on the accumulation of N in the wood samples of the tree species in the present investigation clearly shows that highly significant reductions in N accumulation were found in the polluted samples of M. indica and E. citriodora except in the winter samples of latter in which the difference was only marginal. D. sissoo too experienced severe losses in its N contents in the winter season. On the other hand, significantly higher accumulation of N was observed in the monsoon and winter samples of S. sumini as well as in the summer and winter samples of I. grandis collected from the test site. The trend in the change of concentration was similar in both control and polluted samples of all the investigated species. Further, M. indica and E. citriodora exhibit the maximum concentration of N in their monsoon samples, whereas, the other species recorded the higher values in their winter samples. The data also shows that in general the concentration of N was found higher in the wood than in the bark samples in all the species investigated in the present study.

5. COAL SMOKE POLLUTION AND PHOSPHORUS ASSIMILATION

5.1. INTRODUCTION

Phosphorus is found in the plants as a constituent of nucleic acids, phospholipids, the coenzymes NAD and NADP and most important as a component of ATP and other high energy compounds. Heavy concentrations of phosphorus are found in the meristematic regions of the actively growing parts, where it is used in the synthesis of nucleoproteins and is also involved through ATP in the activation of amino acids for the protein synthesis. As a constituent of nucleoproteins, it is concerned with cell division and transfer of hereditary characters through chromosomes. As a component of phospholipids eg., lecithin, phosphorus is believed to be present in the cell membrane (Devlin and Witham, 1986). Hydrogen ion (H^+) carriers NAD and NADP play a vital role in Kneb's cycle, glycolysis and pentose cycle. Further, phosphate participation is directly in the photochemical events of photosynthesis through ortho-phosphate and nicotinamide adenine dinucleotide phosphate required for the reduction of "assimilatory power" (Devlin and Witham, 1986). Such essential physiological processes as respiration, nitrogen metabolism, carbohydrate metabolism and fatty acid synthesis are

all dependent on the action of these coenzymes. Phosphorus also increases disease resistance in plants, presumably through normal cell development resulting in vigorous growth (Tamhane et al., 1970). Phosphorus deficiency causes decrease in the rate of protein synthesis and results in the accumulation of carbohydrate and soluble nitrogenous compounds (Hewitt, 1963). It also causes premature leaf fall and restricted root and shoot growth.

Phosphorus is present in the soil in two general forms, organic and inorganic. It is the inorganic form of phosphorus which is available to the plants. However, phosphorus bound in organic compounds is eventually liberated from it through decomposition and is released in the inorganic form that is readily taken up by the plants. Further, phosphorus is absorbed by roots both as monovalent (H_2PO_4^-) and divalent (HPO_4^{--}) anions. The quantity of either ion present in the soil is dependent upon pH of the soil solution. The lower pH favours H_2PO_4^- and higher pH, HPO_4^{--} ions (Devlin and Witham, 1986).

5.2. METHODOLOGY

The samples were digested by following the method of Lindner (1944) (as described in case of N) and phosphorus was estimated by the method given by Fiske and Subbarow (1925). In a 10 ml graduated tube, 5 ml of aliquot of peroxide digested material was taken and 1 ml of Molybdate reagent (Appendix-2.1) was added

carefully, followed by 0.4 ml of Amino-naphthol-sulphonic acid (Appendix-2.2). The colour of the solution turned blue and its volume made upto 10 ml with double distilled water. The solution was shaken well and allowed to stand for 5 minutes for maximum colour development. The solution was transferred to a colorimetric tube and percent transmittance was read at 620 nm, on a "spectronic-20" colorimeter. The standard curve was plotted by using known dilutions of monobasic potassium phosphate solution.

5.3. OBSERVATIONS

Phosphorus in Leaves :-

The observations recorded on the contents of P in different seasons in the foliage of experimental trees and the percent variations between control and polluted samples is summarized in table (11). The data indicates that in two fruit trees M. indica and P. guajava, the P contents exhibit increased levels in early winter, whereas, the foliage of other seasons suffered losses due to pollution stress. However, the variations as compared to control in both the species proved to be statistically non-significant. The P contents in S. cumini recorded a significantly severe loss in the foliage of all seasons except in 15 days old summer samples where the loss was only marginal. The monsoon foliage exhibited a loss of about 45% ($P < 0.01$) which

however increased in early winter where it reached its maximum (65%) ($P < 0.01$) and then declined showing the minimum loss of 33% ($P < 0.05$) in late winter samples. The summer leaves of E. citriodora show highly significant ($P < 0.01$) amount of N accumulation (92%) in the polluted atmosphere, whereas, the foliage of remaining seasons experienced severe losses. Among the samples of various seasons in this species, the minimum percent variation of 22% ($P < 0.05$) was recorded in monsoon however, the severity of loss enhanced in early winter where it reached its maximum 63% ($P < 0.01$) and later on under went a fall in the samples of late winter season (44%) ($P < 0.01$). In case of D. sissoo a significantly ($P < 0.01$) higher amount of accumulation (90%) was observed in early winter samples, whereas, the 15 days old summer leaves experience a loss of about 38% ($P < 0.05$) in SO_2 enriched atmosphere. The foliage of other seasons viz. monsoon and late winter though observe losses in P contents, the variation was non-significant as compared to control. Further, P accumulation was severely affected in early winter samples of I. grandis (26%) ($P < 0.05$), whereas, the foliage of other seasons did not record any critical variations as compared to their controls.

It is evident from the data that in all the species investigated in the present study the concentration of P in the leaves of different seasons was maximum in monsoon. While in three species M. indica, P. guajava and I. grandis there was a

Table-11:- Data on the amount of phosphorus (percent dry weight) in the foliage of various tree species in different seasons.

Species	Site	Summer	Monsoon	Early Winter	Late Winter
<u>M. indica</u>	P	0.250±0.033	0.325±0.024	0.290±0.020	0.195±0.021
	C	0.270±0.044 (-7) ^{NS}	0.375±0.049 (-13) ^{NS}	0.250±0.035 (+16) ^{NS}	0.230±0.027 (-15) ^{NS}
<u>P. guajava</u>	P	0.280±0.013	0.300±0.063	0.260±0.039	0.130±0.033
	C	0.320±0.033 (-12) ^{NS}	0.370±0.056 (-19) ^{NS}	0.240±0.057 (+8) ^{NS}	0.135±0.029 (-4) ^{NS}
<u>S. cumini</u>	P	0.200±0.053	0.210±0.014	0.075±0.016	0.170±0.052
	C	0.250±0.063 (-20) ^{NS}	0.380±0.087 (-45) ^{**}	0.215±0.020 (-65) ^{**}	0.255±0.033 (-33) [*]
<u>E. citriodora</u>	P	0.240±0.034	0.300±0.060	0.105±0.015	0.200±0.060
	C	0.125±0.025 (+92) ^{**}	0.385±0.054 (-22) [*]	0.285±0.047 (-63) ^{**}	0.355±0.046 (-44) ^{**}
<u>D. sissoo</u>	P	0.120±0.022	0.340±0.026	0.190±0.014	0.240±0.029
	C	0.195±0.058 (-38) [*]	0.380±0.046 (-11) ^{NS}	0.100±0.015 (+90) ^{**}	0.300±0.062 (-20) ^{NS}
<u>I. grandis</u>	P	0.230±0.032	0.350±0.043	0.255±0.050	0.140±0.040
	C	0.200±0.054 (+15) ^{NS}	0.390±0.057 (-10) ^{NS}	0.345±0.049 (-26) [*]	0.120±0.024 (+17) ^{NS}

Values are mean ± standard deviation.

P = Polluted, C = Control

** = Significant at P<0.01 level

* = Significant at P<0.05 level

NS = Non-significant

(+) and (-) indicate percent increase and decrease over control respectively

gradual decline (after monsoon) as the season proceeds, the rest of the species observe a fall at early winter followed by an increase in late winter samples. Among all the species investigated in the present study, the lowest P contents were observed in the polluted samples of S. cumini in early winter (0.075%) and the maximum in the control monsoon samples of I. grandis (0.390%).

Phosphorus in Bark :-

The data recorded (table-12) on the seasonal variations of P contents in the bark samples of different experimental trees indicates that P. guajava experienced severe losses in the monsoon and winter seasons in the polluted environment showing a reduction of 25% ($P < 0.05$) and 33% ($P < 0.01$) respectively. The other two species E. citriodora and I. grandis also exhibited highly significant ($P < 0.01$) losses in their P contents in all seasons, the magnitude of decline ranging from 29%-38% in the former and 27%-44% in the latter species. On the other hand, M. indica and D. sissoo have shown increased and decreased P levels respectively in their polluted samples but the difference with respect to control was statistically non-significant. Contrary to the behaviour of other species, S. cumini recorded a highly significant ($P < 0.01$) increase (50%) in P contents in its summer samples collected from the population growing at the test site. Whereas, the samples collected in other seasons did not show

Table-12:- Seasonal changes in the amount of phosphorus (percent dry weight) in the bark samples of various tree species.

Species	Site	Summer	Monsoon	Winter
<u>M. indica</u>	P	0.100±0.034	0.110±0.031	0.180±0.050
	C	0.080±0.010 (+25) ^{NS}	0.090±0.023 (+22) ^{NS}	0.150±0.032 (+20) ^{NS}
<u>P. guajava</u>	P	0.070±0.017	0.090±0.007	0.040±0.005
	C	0.070±0.022 (00)	0.120±0.026 (-25) [*]	0.060±0.003 (-33) ^{**}
<u>S. cumini</u>	P	0.090±0.007	0.120±0.028	0.080±0.014
	C	0.060±0.011 (+50) ^{**}	0.110±0.019 (+9) ^{NS}	0.070±0.015 (+14) ^{NS}
<u>E. citriodora</u>	P	0.050±0.004	0.100±0.007	0.080±0.010
	C	0.070±0.007 (-29) ^{**}	0.140±0.019 (-29) ^{**}	0.130±0.014 (-38) ^{**}
<u>D. sissoo</u>	P	0.060±0.017	0.130±0.019	0.080±0.015
	C	0.070±0.014 (-14) ^{NS}	0.150±0.036 (-13) ^{NS}	0.100±0.014 (-20) ^{NS}
<u>I. grandis</u>	P	0.170±0.017	0.180±0.047	0.270±0.044
	C	0.300±0.028 (-43) ^{**}	0.320±0.046 (-44) ^{**}	0.370±0.017 (-27) ^{**}

Values are mean ± standard deviation.

P = Polluted, C = Control

** = Significant at P(0.01 level)

* = Significant at P(0.05 level)

NS = Non-significant

(+) and (-) indicate percent increase and decrease over control respectively

any marked change in their P contents in the same environmental set-up. Though the concentration of P was variable in all the investigated species, the pattern of change of P in different seasons in both the polluted and control samples of a particular species show analogy. Except in M. indica and T. grandis which recorded the highest P levels in their winter samples, the other ones observe the maximum concentration of P in the monsoon season.

Phosphorus in Wood :-

The observations recorded (table-13) on the amount of P in the wood samples of the species investigated in the present study show that in M. indica and T. grandis the concentration of P was significantly ($P < 0.01$) higher in the samples collected from the polluted atmosphere. The increase being 68% and 53% in summer, 62% to 67% in monsoon and 56% and 65% in winter season for M. indica and T. grandis respectively. S. cumini too accumulated marked levels of P in its winter samples in the SO_2 enriched atmosphere, the increase being 50% ($P < 0.01$) over control. On the other hand, pollution caused marked reductions in P levels in monsoon and winter samples of P. quajava experiencing a loss of 36% and 44% respectively. The other two species viz. E. citriodora and D. sissoo also observed significantly ($P < 0.01$) lesser P levels (43% and 36% respectively) in their winter samples collected from the population growing in the abnormal

Table-13:- Seasonal alterations in the phosphorus contents (percent dry weight) in the wood samples of various tree species.

Species	Site	Summer	Monsoon	Winter
<u>M. indica</u>	P	0.320±0.040	0.260±0.049	0.560±0.040
	C	0.190±0.020 (+68)**	0.160±0.028 (+62)**	0.360±0.042 (+56)**
<u>P. guajava</u>	P	0.170±0.022	0.180±0.024	0.150±0.014
	C	0.170±0.014 (00)	0.280±0.050 (-36)**	0.270±0.054 (-44)**
<u>S. cumini</u>	P	0.180±0.013	0.300±0.032	0.150±0.019
	C	0.160±0.028 (+12) ^{NS}	0.300±0.058 (00)	0.100±0.011 (+50)**
<u>E. citriodora</u>	P	0.050±0.013	0.170±0.010	0.080±0.014
	C	0.060±0.010 (-17) ^{NS}	0.200±0.040 (-15) ^{NS}	0.140±0.025 (-43)**
<u>D. sissoo</u>	P	0.170±0.044	0.420±0.022	0.140±0.024
	C	0.200±0.036 (-15) ^{NS}	0.510±0.026 (-18) ^{NS}	0.220±0.022 (-36)**
<u>I. grandis</u>	P	0.520±0.046	0.720±0.064	0.660±0.066
	C	0.340±0.045 (+53)**	0.430±0.019 (+67)**	0.400±0.026 (+65)**

Values are mean ± standard deviation.

P = Polluted, C = Control

** = Significant at P<0.01 level

NS = Non-significant

(+) and (-) indicate percent increase and decrease over control respectively

atmosphere. Moreover, all the investigated species recorded the maximum P concentration in monsoon season except M. indica which observes the highest concentration in its winter samples as compared to other seasons. Further, the trend of change of concentration was similar in polluted and control samples. Interestingly, in general all the species recorded higher amount of P in their wood samples as compared to bark.

5.4. D I S C U S S I O N

Phosphorus in Leaves :-

The data presented in table (11) shows the concentration of phosphorus in the leaves of different seasons in the various tree species undertaken for investigation in the present study. As it is evident from the data that in general all the species growing in SO₂ enriched atmosphere showed losses in the P concentration in various seasons except in the summer and early winter foliage of E. citriodora and D. sissoo respectively, which however, recorded highly significant increases in P accumulation under stress. Out of the six species investigated in the present study, S. cumini and E. citriodora experienced highly significant reductions in P accumulation in all seasons (except in their summer samples), the loss being 33%-65% in the former and 22%-63% in the latter. The other two species D. sissoo and I. grandis also observed marked reduction in P

contents in their summer and early winter foliage respectively. The reductions in the phosphorus levels in the foliage of SO_2 exposed plants have also been reported earlier in Triticum aestivum (Pandey and Rao, 1978); Arachis hypogaea (Mishra, 1980); Triticum aestivum (Prasad, 1980); Pinus sativa (Garsed et al., 1981); Cicer arietinum, Oryza sativa, Panicum miliaceum, Vicia faba (Agrawal, 1982); Diospyros melanoxylon, Lagerstroemia parviflora and Zizyphus nummularia (Pandey, 1983); Festuca arundinaceae (Flegler and Youngner, 1985); Fagus sylvatica (Zech, 1990/91). The decreased P concentrations in the plants growing under SO_2 pollution stress in the present study may be due to the inhibition in certain important enzymatic activities involved in P metabolism. Further, it is expected that plants under stress physiological conditions are likely to loose much energy to combat the hazards. It, therefore, opens a challenge to the pollution problem indicating the possibility of management of pollution stress through manipulation of P balance in the involved system. Moreover, the nutrient supplying capacity of the soil with respect to P seems to be less (table-4) as also reported earlier by Agrawal et al. (1985). Fried and Broeshart (1967) put forth their hypothesis, that SO_2 induced acidity may cause greater solubility and mobility of soil constituents particularly P which may leach from the rooting zone and become unavailable for plant growth.

On the other hand significantly higher P accumulation in the summer leaves of E. citriodora and early winter samples of D. sissoo signifies its lesser utilization in these periods. This may be attributed to the lower rate of metabolic activity as well as the reduced growth performance of the plants in SO₂ polluted atmosphere. Saxe (1983) has also found in Phaseolus vulgaris L. cv. processer an increased concentration of P after being exposed to higher concentration of 250 μgm^{-3} SO₂ for 4-5 weeks.

The data further shows that despite the changes in the amount of P in the leaves in various seasons, the trend was the same in the polluted as well as control samples. Interestingly in all the species investigated in this study, the highest concentration of P was observed in monsoon season regardless of the site of collection, thus showing their peak growth performance with respect to P metabolism. While, in three species, M. indica, P. quajava and I. grandis the P concentration declined as the season advances (from monsoon onwards), the others (S. cumini, D. sissoo and E. citriodora) experience a loss in early winter followed by a gain in late winter season. The loss in the P contents in the foliage after attaining a peak concentration at certain growth stage has also been earlier reported by Guha and Mitchell (1966) in certain deciduous trees and by Lal and Ambasht (1982) in Psidium quajava. This indicates that P might have translocated to other

fast growing organs, which therefore, seems to be an adaptation developed by the plant to restrict any major P loss at the time of leaf fall.

Phosphorus in Bark :-

The concentration of phosphorus recorded in the present study in different bark samples collected in various seasons indicates that its amount was variable among different species (table-12). The lowest concentration (0.040%) was recorded in the polluted samples of P. guajava and the highest (0.370%) in the control samples of I. grandis both collected in winter season. Highly significant reductions were observed in the E. citriodora and I. grandis in all seasons as compared to control, whereas P. guajava recorded the severe losses only in the monsoon and winter samples of the polluted population. On the other hand, a marked increase of about 50% in P accumulation was observed in the summer samples of S. cumini at the same site. However, P contents in M. indica and D. sissoo did not show any significant difference between control and polluted samples. The concentration of P followed the same pattern in all the polluted and control samples of the concerned species. Further, in M. indica and I. grandis there is a steady increase in P concentration from summer to winter, whereas, in rest of the species viz. P. guajava, S. cumini, E. citriodora and D. sissoo,

the highest concentration was observed in the monsoon samples followed by a fall in winter.

Phosphorus in Wood :-

As compared to the concentration of phosphorus in the bark, the wood samples showed higher amounts in all the species investigated in the present study (table-13). Interestingly in the wood of I. grandis the P concentration was higher even more than what was recorded in its foliage. The winter samples of M. indica as well as the summer and monsoon samples of D. sissoo also recorded richer amount of P in their wood compared to their leaves. Since very little amount of P is utilized in the foliage, it seems that the wood parenchyma acts as a store house to accumulate any excess amount which enters into the plant body through roots. Amongst the various species the minimum concentration of P in the wood was observed in the summer samples of E. citriodora (0.050%) and the highest in the monsoon samples of I. grandis (0.720%) both collected from the test site.

The data regarding the percent variations between control and polluted samples shows that highly significant increase was observed in all the samples of M. indica and I. grandis as well as in the winter samples of S. cumini, collected from the population growing in the SO₂ enriched atmosphere. On the other hand, marked reductions in P contents were observed in

the monsoon and winter samples of P. guajava as well as in the winter samples of E. citriodora and D. sissoo of the same site. Further, all the species except M. indica recorded the highest P concentrations in the monsoon season, both in the samples collected from polluted as well as normal sites.

6. COAL SMOKE POLLUTION AND POTASSIUM BALANCE

6.1 INTRODUCTION

The potassium is required in large amounts for plants and, unlike nitrogen and phosphorous, it does not form a stable structural part of any molecule in a plant cell. The highest concentrations of potassium are found in the meristematic regions of the plants. Potassium is essential for the activation of the enzymes and is involved in the synthesis of certain peptide bonds and in carbohydrate metabolism. It also enhances the incorporation of amino acids into proteins (Webster, 1956). The enzymes that require K as an activator include fructokinase, pyruvic acid kinase and transacetylase (Nason and McElroy, 1963). K is essential for some vital metabolic processes including glycolysis, oxidative phosphorylation and adenine synthesis (Evans and Sorger, 1966). It is actively involved in the translocation of solutes moving across the sieve plates by electro-osmosis (Salisbury and Ross, 1986). Its role is inevitable in the physiological processes like photosynthesis, chlorophyll development and the water balance of the leaves. The best known function of the K is its role in stomatal opening and closing (Fischer and Hsiao, 1968; Humble and Hsiao, 1969). Its

deficiency causes necrosis, rosette or bushy habit of growth and weakening of stems and decreases the resistance against pathogens.

Potassium is present in the soil in a non-exchangeable (fixed) form, an exchangeable form, and a soluble form, which always remain in equilibrium and a change in the concentration of any one of the constituents will cause a shift towards stabilization. For example, depletion of the soluble K in the soil by the plant and soil micro-organisms will cause a release of exchangeable K, which in turn will cause the slow release of fixed K. This situation is desirable because adsorbed and fixed K which is not readily leached from the soil can be made available to the plants.

6.2. METHODOLOGY

The samples of leaves, bark and wood were digested by the method of Lindner, 1944 (as described in case of N). The potassium contents in the samples were estimated flame photometrically. One ml of aliquot (peroxide digested material) was suitably diluted with double distilled water in a graduated tube. A blank containing only distilled water was run simultaneously. The readings were compared with a calibration curve plotted using the known dilutions of a standard potassium sulphate solution. The potassium was expressed on percent basis.

6.3. OBSERVATIONS

Potassium in Leaves :-

The data given in table (14) clearly indicates the trend of potassium balance in the different species investigated and also the percent variations between control and polluted samples. The observations recorded reveals that pollution caused a uniform K depletion in the foliage of all the experimental species with a single exception of T. grandis. The data clearly shows that in M. indica the magnitude of loss was lower in 15 days old summer leaves (18%) ($P < 0.05$) and the severity of the loss increased in the following seasons and the maximum reduction was observed in late winter (48%) ($P < 0.01$). The polluted samples of P. guajava also record reduced values of K as in M. indica, however, the degree of loss was less as well as statistically non-significant. In S. cumini K levels also suffered a set-back. While the loss was non-significant in the summer leaves, the foliage of other seasons experience marked reductions in the polluted atmosphere. The loss was maximum (25%) ($P < 0.01$) in monsoon, whereas, early and late winter samples respond equally experiencing the same level of stress (20%) ($P < 0.05$). The trend of K loss in the foliage of E. citriodora and D. sissoo was almost similar in all seasons, although, the 15 days old summer leaves of the former exhibit a highly significant ($P < 0.01$) loss (31%), while in the latter the reduction was comparatively low

showing a variation of only 12% ($P < 0.05$). In both these species the K levels progressively decreased in polluted atmosphere as the season advanced recording the maximum loss of 43% in E. citriodora and 39% in D. sissoo ($P < 0.01$) in early winter. The percent variation in the K accumulation however, observed a fall in late winter samples of both the species in the same environmental set-up. In contrast to the behaviour of other species, I. grandis observes a highly significant ($P < 0.01$) increase of K in its foliage of all seasons except in late winter where the difference was non-critical. The highest increase in K accumulation was observed in the early winter leaves (44%) of the population growing at the polluted site as compared to the foliage of the same season collected from the trees growing at the reference site.

The concentration of K in the foliage of different seasons exhibited varied trends in the different experimental species (table-14). In M. indica the K content was recorded the highest in summer which then declined in monsoon season. The K contents again raised in early winter followed by a fall in late winter samples. In P. guajava and D. sissoo after the highest concentration recorded in the 15 days old summer samples, there is a steady decline in K concentration as the season proceeded till it shows its minimum amount in late winter samples. The trend was however different in S. cumini. Here K contents observed an initial fall in monsoon season where upon the

Table-14:- Data on the amount of potassium (percent dry weight) in the foliage of various tree species in different seasons.

Species	Site	Summer	Monsoon	Early Winter	Late Winter
<u>M. indica</u>	P	1.040±0.132	0.650±0.100	0.695±0.130	0.450±0.070
	C	1.265±0.133 (-18)*	0.920±0.124 (-29)**	1.020±0.142 (-32)**	0.860±0.092 (-48)**
<u>P. guajava</u>	P	1.235±0.133	0.970±0.152	0.945±0.116	0.790±0.068
	C	1.355±0.131 (-9) ^{NS}	1.170±0.158 (-17) ^{NS}	1.090±0.132 (-13) ^{NS}	0.920±0.136 (-14) ^{NS}
<u>S. cumini</u>	P	0.970±0.093	0.650±0.070	0.780±0.080	0.860±0.073
	C	1.090±0.141 (-11) ^{NS}	0.870±0.093 (-25)**	0.970±0.141 (-20)*	1.080±0.136 (-20)*
<u>E. citriodora</u>	P	1.140±0.134	1.170±0.093	0.625±0.050	0.595±0.089
	C	1.660±0.132 (-31)**	1.750±0.114 (-33)**	1.090±0.085 (-43)**	0.940±0.104 (-37)**
<u>D. sissoo</u>	P	1.610±0.138	0.960±0.096	0.810±0.076	0.720±0.082
	C	1.840±0.153 (-12)*	1.415±0.154 (-32)**	1.335±0.122 (-39)**	1.040±0.104 (-31)**
<u>I. grandis</u>	P	1.380±0.141	2.000±0.158	1.120±0.146	0.525±0.050
	C	1.090±0.085 (+27)**	1.650±0.100 (+21)**	0.780±0.080 (+44)**	0.455±0.064 (+15) ^{NS}

Values are mean ± standard deviation.

P = Polluted, C = Control

** = Significant at P<0.01 level

* = Significant at P<0.05 level

NS = Non-significant

(+) and (-) indicate percent increase and decrease over control respectively

concentration gradually increased with the advancement of season. The change in the concentration of K in the foliage of E. citriodora and I. grandis of both the sites followed the same trend. After an initial increase the maximum K contents were found in monsoon season followed by a steady fall till the concentration touched its minimum in the late winter samples.

Potassium in Bark :-

The analysis of K in the bark samples of various experimental tree species reveals (table-15) that the population of M. indica growing at the test site experienced severe losses in its K contents showing 41, 36, and 40 percent variations ($P < 0.01$) over control in summer, monsoon and winter seasons respectively. P. guajava also observed highly significant ($P < 0.01$) losses in its summer and winter samples, the magnitude of loss being quite high (49%) in the former compared to the latter season (29%). While K levels in S. cumini and E. citriodora did not differ significantly in summer season, there was a marked ($P < 0.01$) increase in loss ranging from 45%-71% in the former and 72%-74% in the latter in their monsoon and winter samples respectively. D. sissoo and I. grandis were the only species to record significantly high K levels in the polluted samples of all seasons except in the monsoon samples of the latter where the difference compared to control on statistical analysis proved to be non-significant. Both the species, however, exhibited the

Table-15:- Seasonal changes in the potassium contents (percent dry weight) in the bark samples of various tree species.

Species	Site	Summer	Monsoon	Winter
<u>M. indica</u>	P	0.400±0.045	0.550±0.059	0.420±0.052
	C	0.680±0.068 (-41)**	0.860±0.073 (-36)**	0.700±0.130 (-40)**
<u>P. quajava</u>	P	0.360±0.073	0.780±0.080	0.550±0.036
	C	0.700±0.070 (-49)**	0.850±0.100 (-8) ^{NS}	0.780±0.059 (-29)**
<u>S. cumini</u>	P	0.200±0.034	0.460±0.073	0.230±0.066
	C	0.230±0.049 (-13) ^{NS}	0.840±0.108 (-45)**	0.800±0.079 (-71)**
<u>E. citriodora</u>	P	0.150±0.034	0.260±0.064	0.180±0.028
	C	0.180±0.032 (-17) ^{NS}	0.920±0.059 (-72)**	0.690±0.132 (-74)**
<u>D. sissoo</u>	P	0.640±0.045	0.840±0.096	0.780±0.063
	C	0.500±0.059 (+28)**	0.680±0.068 (+24)**	0.670±0.046 (+16)*
<u>I. grandis</u>	P	0.640±0.068	0.700±0.036	0.670±0.063
	C	0.470±0.046 (+36)**	0.680±0.038 (+3) ^{NS}	0.540±0.053 (+24)**

Values are mean ± standard deviation.

P = Polluted, C = Control

** = Significant at P(0.01 level)

* = Significant at P(0.05 level)

NS = Non-significant

(+) and (-) indicate percent increase and decrease over control respectively

maximum increase ($P<0.01$) in K contents in the summer season showing a variation of 28% in D. sissoo and 36% in I. grandis.

The concentration of K in the bark samples though showed varied amounts in different seasons, the trend of change in the concentration was similar in control and polluted samples of a particular species. Invariably in all the investigated species, the minimum K levels were observed in summer samples and the maximum concentration in the samples collected in monsoon season. In general, all the species observed higher value of K contents in their bark samples compared to wood.

Potassium in Wood :-

The observations on the K contents in the wood samples of the species undertaken for investigation in the present study (table-16) indicate that in M. indica the amount of K was low in all seasons, although, the variation was highly significant ($P<0.01$) in winter season exhibiting a loss of about 39% compared to control. The populations of P. guajava, S. cumini as well as E. citriodora showed highly significant ($P<0.01$) reductions in K concentration in the polluted atmosphere in all the seasons. The losses ranged from 52-54% in P. guajava, 33-52% in S. cumini and 37-48% in E. citriodora. D. sissoo was the only species which did not record any significant losses in its polluted samples. On the other hand, I. grandis stood alone of its type to exhibit significant ($P<0.01$) increase in K levels in

Table-16:- Seasonal alterations in the potassium contents (percent dry weight) in the wood samples of different tree species.

Species	Site	Summer	Monsoon	Winter
<u>M. indica</u>	P	0.395±0.036	0.440±0.040	0.280±0.050
	C	0.410±0.040	0.500±0.049	0.460±0.073
		(-4) ^{NS}	(-12) ^{NS}	(-39) ^{**}
<u>P. guajava</u>	P	0.120±0.013	0.260±0.040	0.240±0.010
	C	0.260±0.013	0.550±0.036	0.500±0.080
		(-54) ^{**}	(-53) ^{**}	(-52) ^{**}
<u>S. cumini</u>	P	0.120±0.026	0.200±0.046	0.140±0.013
	C	0.250±0.059	0.300±0.040	0.230±0.011
		(-52) ^{**}	(-33) ^{**}	(-39) ^{**}
<u>E. citriodora</u>	P	0.100±0.026	0.160±0.038	0.120±0.013
	C	0.160±0.025	0.310±0.013	0.230±0.020
		(-37) ^{**}	(-48) ^{**}	(-48) ^{**}
<u>D. sissoo</u>	P	0.180±0.022	0.300±0.028	0.330±0.042
	C	0.200±0.059	0.360±0.040	0.370±0.044
		(-10) ^{NS}	(-17) ^{NS}	(-11) ^{NS}
<u>I. grandis</u>	P	0.250±0.034	0.260±0.034	0.360±0.042
	C	0.150±0.013	0.220±0.042	0.250±0.026
		(+67) ^{**}	(+18) ^{NS}	(+44) ^{**}

Values are mean ± standard deviation.

P = Polluted, C = Control

** = Significant at P<0.01 level

NS = Non-significant

(+) and (-) indicate percent increase and decrease over control respectively

its summer (67%) and winter samples (44%) compared to control. However, the K levels in the monsoon season did not differ significantly in control and polluted samples.

The trend in the change of K concentration in different seasons in a particular species showed close similarity in the control and polluted samples. Moreover, except D. sissoo and I. grandis which record the highest K contents in the winter season, the rest of the species investigated in the present study observed the maximum amounts in monsoon samples.

6.4. D I S C U S S I O N

Potassium in Leaves :-

The data on the seasonal variations in K contents in the foliage of different tree species is presented in table (14). It is quite evident from the observations that in all the species the concentration of K is higher in control leaves except in I. grandis which however recorded the higher values in the samples of the polluted environment. Under the SO₂ enriched atmosphere the species like M. indica, E. citriodora and D. sissoo exhibited highly significant losses in their K contents in all seasons. S. cumini also recorded appreciably reduced K values in the foliage of all seasons except in 15 days old summer samples which show a non-significant loss under pollution stress. Several other authors in the past have reported decreased K

contents in the plants after having been exposed to SO_2 (Prasad, 1980, Agrawal, 1982). On the other hand P. guajava did not experience any significant variations in its K contents in the polluted environment. The behaviour of I. grandis was found to be quite unique which shows a positive response with regard to K accumulation observing an increase of 15% to 44% in its foliage of the polluted environment compared to control. A similar increase in K levels in the plants exposed to SO_2 pollution was observed by Saxe (1983) and Pandey (1983).

Among the various species the highest K concentration was observed in monsoon samples of I. grandis (2.00%) and the lowest in the late winter samples of M. indica (0.450%), both collected from the polluted site. Although the concentration of K was variable in the foliage in different seasons, the trend was the same in polluted as well as control plants. Moreover, out of six species investigated in the present study 4 species viz. M. indica, P. guajava, S. cumini and D. sissoo recorded the maximum K concentrations in their 15 days old summer samples, whereas, the other two species, E. citriodora and I. grandis observe the peak values in the monsoon season.

Potassium in Bark :-

The analysis of K in the bark samples collected in various seasons from different tree species, both growing at the test site as well as at the normal sites (table-15) reveals that

there was a loss in K level in the polluted samples of M. indica, P. guajava, S. cumini and E. citriodora compared to control. On the other hand, in D. sissoo and I. grandis significantly higher K contents were observed in all the seasons except in the monsoon samples of latter where the difference proved to be only marginal. Further, the maximum percent reduction in K was observed in the winter samples of E. citriodora (74%) and the minimum in the monsoon samples of P. guajava (8%) compared to control. The observations recorded on the concentration of K in different seasons in various species shows that the minimum concentration (0.150%) was observed in the summer samples of E. citriodora and also the maximum (0.920%) in the monsoon samples of same species, the former collected from the trees growing at the test site and the latter from the normal site. Interestingly in all the species the K concentration was higher in the monsoon season in both polluted and control samples.

Potassium in Wood :-

The contents of K in wood samples of the trees undertaken for investigation in the present study (table-16) show reduced levels in all the species in SO₂ enriched environment except in I. grandis which recorded higher amounts. Among the various species the concentration of K was the highest in the monsoon samples of P. guajava (0.550%) and the least in the summer

samples of E. citriodora (0.100%), the former collected from the control and the latter from the polluted site. The trend in the variation of K concentration was the same in polluted as well as control samples for a particular species. The highest concentration of K among the various samples was observed in the monsoon season in case of M. indica, P. quajava, S. cumini and E. citriodora. The other two species viz. D. sissoo and I. grandis, recorded the highest concentration of K in their winter samples. The data on the percent variations in control and polluted samples shows that in SO₂ enriched atmosphere P. quajava experienced the maximum loss of about 54% whereas, the minimum loss was observed in the polluted samples of M. indica (4%), both collected in the summer season.

It is clear from the data that I. grandis was the unique species which shows elevated levels of K in its foliage, bark as well as wood samples in the polluted environment. On the other hand, rest of the species exhibit decreased amounts of K except in the bark samples of D. sissoo which shows significantly elevated concentration in the polluted atmosphere. Moreover, all the species, in general, recorded higher amounts of K in their bark samples compared to wood, regardless of the site of collection.

7. COAL SMOKE POLLUTION AND PHOTOSYNTHETIC PIGMENTS

7.1. INTRODUCTION

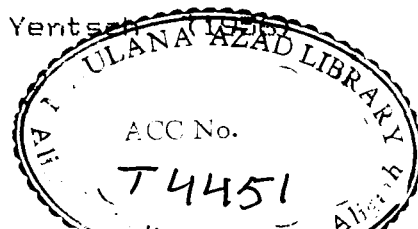
Chlorophylls, the green pigments of plants are the most important pigments responsible for the conversion of light energy into chemical energy and are thus active in the process of photosynthesis. Chlorophyll a and b are the most abundant pigments in higher plants. Chlorophyll molecule has a cyclic tetrapyrrolic structure (porphyrin), with an isocyclic ring containing a magnesium atom at its centre and a phytol chain attached to it. Carotenoids, on the other hand are also found in varying amounts in nearly all higher plants and are believed to be vital for two important functions (a), they protect against the photo-oxidation of chlorophyll and (b), they absorb and transfer light energy to chlorophyll a (Devlin and Witham, 1986).

Sulphur dioxide is known to interact with the metabolism of photosynthetic pigments in plants. Acute and chronic exposure to SO_2 can result in the general disruption of photosynthesis and respiration as well as other metabolic and fundamental cellular processes (Ewald and Schlee, 1983). The chemical reaction of SO_2 with plants is primarily due to its

acidic nature which in solution disturbs both mineral status of the plant and its physiological processes (Thomas, 1961). Degradation of chlorophyll to phaeophytin has been demonstrated by Rao and LeBlanc (1966) and Syrett and Warstell (1969) in lichens and bryophytes.

7.2. METHODOLOGY

The fresh leaf samples after being brought to the laboratory were removed from the ice bags and their surface gently cleaned with the moist cotton to remove any particulate matter deposited over them. The chlorophyll content was estimated by following the method of Arnon (1949). One gram fresh sample was crushed gently with 80% acetone in a mortar and pestle. To this was added a little pinch of calcium carbonate (CaCO_3). The samples after being ground to a fine pulp were centrifuged (5000 rpm for 5 minutes) and the supernatant transferred to a 100 ml volumetric flask. The residue was repeatedly ground and centrifuged till it turned colourless, thus ensuring the complete extraction of chlorophyll from the tissue. The volume of the extract was made 100 ml by adding 80% acetone. The absorption of the solution was read at 663, 645, 510 and 480 nm on spectrophotometer. The chlorophylls (a and b) and carotenoid contents were analysed by applying the formulae given by MacLachlan and Zalik (1963) and Duxbury and Yentsch (1957).



respectively. The total chlorophyll was estimated by applying the formula given by Arnon (1949).

$$\text{mg chlorophyll a/g tissue} = \frac{12.3 D_{663} - 0.86 D_{645}}{d \times 1000 \times W} \times V$$

$$\text{mg chlorophyll b/g tissue} = \frac{19.3 D_{645} - 3.60 D_{663}}{d \times 1000 \times W} \times V$$

$$\text{mg carotenoids /g tissue} = \frac{7.6 D_{480} - 1.49 D_{510}}{d \times 1000 \times W} \times V$$

$$\text{mg total chlorophyll/g tissue} = \frac{20.2 D_{645} + 8.02 D_{663}}{d \times 1000 \times W} \times V$$

Where D_{663} , D_{645} , D_{510} and D_{480} represent the values of optical densities at the respective absorption spectra.

V = final volume of chlorophyll extract in 80% acetone.

W = fresh weight of tissue extracted.

d = length of the light path.

7.3. OBSERVATIONS

The data available on the impact of coal smoke pollution on the photosynthetic pigments in the leaves of the various tree species in the present investigation reveals that in general the chlorophyll pigments as well as carotenoids suffered heavy losses in the population growing in the polluted environment. It is also clear from the data that universally all the

investigated species exhibited the higher losses in chlorophyll a compared to chlorophyll b.

In M. indica (table-17) the losses observed in the chlorophyll a were maximum in 15 days old summer leaves (33%) ($P<0.01$) whereas, in the foliage collected in the following seasons, the magnitude of loss reduced to half showing a variation of 14% and 16% ($P<0.05$) in monsoon and early winter samples respectively. chlorophyll b in this species in all the seasons did not show any marked difference compared to control. On the other hand, a significant loss was observed in the total chlorophyll in all the seasons, the maximum (24%) ($P<0.01$) being recorded in 15 days old summer leaves and the least (10%) ($P<0.05$) in the samples of monsoon season.

The amount of chlorophyll a and b in case of P. guajava recorded a significant decrease in summer and monsoon seasons, whereas, the samples collected in the early winter did not show any marked difference in the polluted atmosphere (table-18). While the chlorophyll a experienced maximum loss (35%) ($P<0.01$) in 15 days old summer leaves, the chlorophyll b was reduced highly in monsoon samples (25%) ($P<0.01$). Similarly, total chlorophyll was decreased almost to the same level in both summer and monsoon samples, showing a variation of 27% and 28% respectively. Carotenoid contents though exhibit severe ($P<0.01$) losses in 15 days old summer leaves (15%) and early winter

Table-17:- Data on seasonal changes in the chlorophyll and carotenoid contents (mg/g fresh weight) of *M. indica* growing at different sites.

Parameter	Site	Summer	Monsoon	Early Winter
Chlorophyll a	P	0.325±0.010	1.688±0.186	0.510±0.068
	C	0.486±0.008	1.963±0.144	0.604±0.059
		(-33)**	(-14)*	(-16)*
Chlorophyll b	P	0.274±0.027	1.370±0.171	0.396±0.068
	C	0.298±0.043	1.440±0.091	0.430±0.058
		(-8)NS	(-5)NS	(-8)NS
Carotenoid	P	0.173±0.005	0.649±0.035	0.288±0.049
	C	0.178±0.005	0.710±0.044	0.400±0.050
		(-3)NS	(-9)*	(-28)**
Total Chlorophyll	P	0.599±0.028	3.058±0.254	0.906±0.106
	C	0.785±0.049	3.403±0.191	1.034±0.012
		(-24)**	(-10)*	(-12)*

Values are mean ± standard deviation

P = Polluted, C = Control

** = Significant at P<0.01 level

* = Significant at P<0.05 level

NS = Non-significant

(-) indicates percent decrease over control

Table-18:- Data on seasonal changes in the chlorophyll and carotenoid contents (mg/g fresh weight) of *P. puaia* growing at different sites.

Parameter	Site	Summer	Monsoon	Early Winter
Chlorophyll a	P	0.816±0.012	1.305±0.035	0.500±0.094
	C	1.247±0.170 (-35)**	1.847±0.023 (-29)**	0.540±0.077 (-7) ^{NS}
Chlorophyll b	P	0.677±0.011	0.960±0.014	0.370±0.068
	C	0.787±0.029 (-14)**	1.280±0.012 (-25)**	0.390±0.062 (-5) ^{NS}
Carotenoid	P	0.265±0.023	0.579±0.026	0.170±0.040
	C	0.313±0.009 (-15)**	0.613±0.042 (-6) ^{NS}	0.300±0.049 (-43)**
Total Chlorophyll	P	1.494±0.019	2.266±0.047	0.870±0.118
	C	2.035±0.168 (-27)**	3.127±0.019 (-28)**	0.930±0.125 (-6) ^{NS}

Values are mean ± standard deviation

P = Polluted, C = Control

** = Significant at P<0.01 level

NS = Non-significant

(-) indicates percent decrease over control

foliage (43%), the damage recorded in the monsoon season was non-significant.

In S. cumini a highly significant ($P<0.01$) depletion in chlorophyll a, b and total chlorophyll was observed in all the seasons (table-19). The highest percent loss was observed in the early winter samples [(58% for chlorophyll a, 40% for chlorophyll b and 51% for total chlorophyll) ($P<0.01$)]. However, during summer and monsoon, the foliage experienced almost the same level of stress, the percent loss in chlorophyll a being slightly lesser in the former (31%) than in the latter season (33%). On the other hand, carotenoid contents suffered the maximum reduction in summer (43%) ($P<0.01$) and the minimum in monsoon samples (19%) ($P<0.01$) due to pollution stress.

In E. citriodora (table-20), the losses incurred on chlorophyll a ranged from 26%-35% ($P<0.01$). The minimum decrease is being observed in early winter leaves and the maximum in the foliage of monsoon season, whereas, the total chlorophyll suffered ($P<0.01$) to a highest level in monsoon samples (28%) and the lowest in 15 days old summer samples (20%). Chlorophyll b and carotenoids on the other hand, were reduced significantly ($P<0.01$) in early winter samples (24% and 36% respectively), while, the leaves collected in other seasons did not show any marked variation compared to control.

Table-19:- Data on seasonal variations in the chlorophyll and carotenoid contents (mg/g fresh weight) of *S. cumini* growing at different sites.

Parameter	Site	Summer	Monsoon	Winter
Chlorophyll a	P	0.531±0.009	1.104±0.016	0.215±0.024
	C	0.768±0.010	1.646±0.070	0.507±0.038
		(-31)**	(-33)**	(-58)**
Chlorophyll b	P	0.238±0.015	0.918±0.014	0.176±0.038
	C	0.300±0.013	1.133±0.023	0.295±0.040
		(-21)**	(-19)**	(-40)**
Carotenoid	P	0.122±0.012	0.261±0.018	0.127±0.020
	C	0.215±0.008	0.320±0.016	0.174±0.026
		(-43)**	(-19)**	(-27)**
Total Chlorophyll	P	0.770±0.024	2.022±0.012	0.391±0.022
	C	1.068±0.021	2.779±0.093	0.802±0.108
		(-28)**	(-27)**	(-51)**

Values are mean ± standard deviation

P = Polluted, C = Control

** = Significant at P<0.01 level

(-) indicates percent decrease over control

Table-20:- Data on seasonal variations in the chlorophyll and carotenoid contents (mg/g fresh weight) of *E. citriodora* growing at different sites.

Parameter	Site	Summer	Monsoon	Early Winter
Chlorophyll a	P	0.398±0.017	0.854±0.009	0.348±0.053
	C	0.549±0.013 (-28)**	1.311±0.077 (-35)**	0.468±0.044 (-26)**
Chlorophyll b	P	0.299±0.059	0.603±0.015	0.250±0.028
	C	0.321±0.037 (-7) NS	0.624±0.016 (-3) NS	0.330±0.021 (-24)**
Carotenoid	P	0.213±0.053	0.475±0.015	0.105±0.020
	C	0.227±0.042 (-6) NS	0.483±0.020 (-2) NS	0.165±0.029 (-36)**
Total Chlorophyll	P	0.698±0.064	1.457±0.021	0.598±0.057
	C	0.870±0.049 (-20)**	2.035±0.083 (-28)**	0.798±0.062 (-25)**

Values are mean ± standard deviation

P = Polluted, C = Control

** = Significant at P<0.01 level

NS = Non-significant

(-) indicates percent decrease over control

The observations (table-21) obtained on the contents of photosynthetic pigments in D. sissoo indicate that the foliage in all seasons (summer, monsoon and early winter), experienced significant ($P<0.01$) reductions in the polluted atmosphere, the magnitude of which was high in 15 days old summer samples (32%-48%) while the lowest percent loss was observed in monsoon samples (15% - 26%).

The inhibitory effect of air pollution on the chlorophyll pigments of I. grandis was also found to be considerable (table-22). The maximum reductions in chlorophyll a, b as well as total chlorophyll were observed in monsoon samples (showing a variation of 39%, 31% and 36% respectively) ($P<0.01$) followed by early winter leaves showing a loss of 33% for chlorophyll a, 24% for chlorophyll b and 30% for total chlorophyll and further, the carotenoid contents also reduced to a significant ($P<0.01$) level in both the seasons referred above, the highest loss being recorded in the early winter (43%) and the minimum in the monsoon season (27%). On the other hand, in 15 days old summer samples significant ($P<0.01$) reductions were observed only in chlorophyll a (32%), while, chlorophyll b and carotenoids did not exhibit any critical change in response to air pollution.

Table-21:- Data on seasonal variations in the chlorophyll and carotenoid contents (mg/g fresh weight) of D. sissoo growing at different sites.

Parameter	Site	Summer	Monsoon	Winter
Chlorophyll a	P	0.502±0.043	1.391±0.037	0.500±0.070
	C	0.926±0.035 (-46)**	1.882±0.058 (-26)**	0.789±0.036 (-37)**
Chlorophyll b	P	0.440±0.005	0.941±0.006	0.407±0.034
	C	0.643±0.026 (-32)**	1.105±0.017 (-15)**	0.521±0.028 (-22)**
Carotenoid	P	0.194±0.017	0.500±0.062	0.160±0.042
	C	0.370±0.016 (-48)**	0.705±0.054 (-17)**	0.260±0.042 (-38)**
Total Chlorophyll	P	0.942±0.040	2.332±0.034	0.907±0.104
	C	1.569±0.054 (-40)**	2.987±0.068 (-22)**	1.310±0.057 (-31)**

Values are mean ± standard deviation

P = Polluted, C = Control

** = Significant at P(0.01 level

(-) indicates percent decrease over control

Table-22:- Data on seasonal variations in the chlorophyll and carotenoid contents (mg/g fresh weight) of T. prandis growing at different sites.

Parameter	Site	Summer	Monsoon	Winter
Chlorophyll a	P	0.310±0.034	0.856±0.020	0.582±0.068
	C	0.455±0.013 (-32) **	1.401±0.009 (-39) **	0.870±0.050 (-33) **
Chlorophyll b	P	0.265±0.010	0.636±0.010	0.380±0.052
	C	0.285±0.018 (-7) NS	0.918±0.009 (-31) **	0.500±0.049 (-24) **
Carotenoid	P	0.165±0.017	0.475±0.015	0.248±0.041
	C	0.180±0.013 (-8) NS	0.647±0.032 (-27) **	0.439±0.031 (-43) **
Total Chlorophyll	P	0.545±0.041	1.493±0.013	0.962±0.040
	C	0.740±0.015 (-26) **	2.319±0.019 (-36) **	1.370±0.013 (-30) **

Values are mean ± standard deviation.

P = Polluted, C = Control

** = Significant at P<0.01 level

NS = Non-significant

(-) indicates percent decrease over control

7.4. D I S C U S S I O N

The data (table 17-22) shows that there is an overall reduction in the photosynthetic pigments in the leaves in all seasons and the degree of loss varied in different species depending upon the time of collection and the genetic constitution of the species. It is clear from the data that out of six species investigated in the present study, two species S. cumini (table-19) and D. sissoo (table-21) experienced highly significant reductions in their photosynthetic pigments in all seasons. While the former species exhibits the maximum loss (27-58%) in its early winter samples, in the latter, the highest degree of decline was observed in 15 days old summer leaves (32-48%). In M. indica, the loss in the total chlorophyll was recorded maximum in 15 days old summer samples (24%), whereas, P. guajava, E. citriodora and T. grandis exhibit severe losses in total chlorophyll content in their monsoon leaves (28%, 28% and 36% respectively). Similar SO_2 induced chlorophyll destruction has been reported earlier by many workers (Malhotra, 1977; Pandey and Rao, 1978; Prasad and Rao, 1982; Devi and Patel, 1983; Pandey, 1983; Irving and Miller, 1984; Malhotra and Khan, 1984; Sharma and Rao, 1985; Singh et al., 1985; Yunus et al., 1985; Kumar and Yadav, 1986; Nandi et al., 1986; Singh and Rao, 1986; Agrawal et al., 1987; Chand and Kumar, 1987; Gupta and Ghose, 1987b; Beg and Farooq, 1988; Farooq et al., 1988; Singh

and Rao, 1988; Vijayan and Bedi, 1988; Prakash *et al.*, 1989a; Singh, 1989; Saquib and Ahmad, 1991; Sharma and Prakash, 1991; Ghouse *et al.*, 1993).

The degradation of chlorophyll pigments in foliage of the species growing at the polluted site may be due to the action of SO_2 on chlorophyll metabolism (Lauenroth and Dodd, 1981) and may be attributed to SO_2 induced removal of Mg^{++} ions by two atoms of hydrogen from chlorophyll molecule which converts chlorophyll into phaeophytin (Rao and LeBlanc, 1966) or by the production of superoxide radicals by the reaction of sulphite with chlorophyll under illumination (Shimazaki *et al.*, 1980). On the other hand, some workers are of the opinion that H^+ , HSO_3^- , SO_3^{-2} and SO_4^{-2} ions, which are produced by SO_2 dissolution in water in the cytoplasm, are preferentially incorporated into thylakoid membranes (Ziegler, 1977) and induce chloroplast swelling (Wellburn, 1972). Further, Malhotra (1977) has proposed another mechanism for the reduction in the leaf chlorophyll following the exposure to SO_2 . Chlorophyll is stabilized by forming a complex with proteins and perhaps SO_2 attacks this complex before the actual breakdown of chlorophyll occurs.

It is also evident from the data that in all the investigated species, chlorophyll a pigments recorded severe losses due to pollution stress compared to chlorophyll b. The higher sensitivity and greater susceptibility of chlorophyll a

to pollution stress as observed in the present study gains support from the findings of several earlier workers (Sunderland, 1967; Malhotra, 1977; Kondo et al., 1980; Choudhry and Sinha, 1982; Periasamy and Vivekanandan, 1982; Prasad and Rao, 1982; Singh et al., 1985; Singh and Rao, 1986; Gupta and Ghose, 1987b; Khan and Usmani, 1988; Prakash et al., 1989b, Singh, 1989). The greater loss of chlorophyll a than b may be ascribed to the inactivation of various enzymes associated with the synthesis and action of chlorophyll a (Khan and Usmani, 1988). The higher sensitivity of chlorophyll a to pollution stress hampers the plant growth considerably as it plays a very important role in the process of photosynthesis (Malhotra, 1977).

The observations recorded also show that carotenoid contents in 15 days old summer samples of M. indica, P. qualava, E. citriodora and I. grandis as well as the monsoon samples of all the investigated species exhibit reduced values in the abnormal atmosphere. The percent reduction values show that the degree of loss was lesser in comparison to the losses incurred on total chlorophylls. This indicates that chlorophyll contents in the foliage in these seasons seems to be more sensitive to SO₂ pollution than carotenoids thus coinciding the findings of Nouchi et al. (1973) and Prasad and Rao (1982). However, in early winter samples, all the species, except S. cumini, experienced significantly higher degrees of losses in

carotenoids than in chlorophylls, thus showing their higher susceptibility to pollution stress. These findings are in conformity with the earlier reports of Ardnt (1971) and Lone et al. (1993).

The data regarding the pigment concentrations reveal that the amount of chlorophylls as well as carotenoids in all the species studied was the highest in monsoon season. Among chlorophyll a and b, the concentration of chlorophyll a was maximum, followed by chlorophyll b and the concentration of carotenoids was the least in all the investigated species.

B. COAL SMOKE POLLUTION AND CARBOHYDRATE METABOLISM

B.1. INTRODUCTION

The carbohydrates are important to the plant in several ways. First, they represent a means for the storage of the energy. Second, they are important constituents of the supporting tissues that enable the plant to achieve erect habit. Third, they provide the carbon skeletons for the organic compounds that make up the plant (Devlin and Witham, 1986).

Plant growth and development represent a complex series of co-ordinated events which are ultimately dependent upon the organic reserves accumulated in the seed (cotyledons) while subsequent growth depends upon the translocation of carbohydrates in excess of their maintenance needs. The productivity of mature leaves, therefore represent an asset to the total carbon economy of a plant while developing leaves, flowers and fruits represent liabilities requiring an input of carbon. In most cases, the productivity of mature leaves is more than sufficient to meet these demands of carbon for growth and development. Exposure to gaseous pollutants can however, alter the balance of a plant's carbon economy so that growth can be retarded and yield reduced. In the case of chronic exposures,

reductions in growth and yield can occur without accompanying visible symptoms of injury.

8.2. METHODOLOGY

The total carbohydrate contents in leaves, bark and wood samples was estimated by following the method of Dubois et al. (1956) (Sadasivam and Manikam, 1992a). 100 mg of the dried sample was taken in a boiling tube and hydrolysed by keeping it in a boiling water bath for three hours with 5 ml of 2.5N-HCl and cooled to room temperature. Solid sodium carbonate was added to this solution to neutralize it until the effervescence ceased. The volume of the solution was made up to 100 ml and then centrifuged. Then 0.1 and 0.2 ml of the sample solution was pipetted out in graduated tubes and final volume made upto 1 ml with double distilled water. 1 ml of 5% phenol solution was added to each tube followed by 5 ml of 96% H₂SO₄ and shaken well. The test tubes with solutions after 10 minutes were then placed in water bath at 25-30°C for 20 minutes and the colour read at 490 nm on "spectronic-20" colorimeter. A blank was run with each sample. The carbohydrate content was calculated by comparing the optical density of the sample with a calibration curve plotted by taking known dilutions of standard solutions of chemically pure glucose.

8.3. OBSERVATIONS

Total Carbohydrate in Leaves :-

The data given in table (23) on the carbohydrate contents in the leaves of experimental trees reveals that in M. indica population growing at the polluted site, reduced levels of carbohydrate were found in the foliage of all seasons. However, the variation compared to control on statistical analysis proved to be non-significant. P. guajava exhibited severe losses (18%) ($P<0.01$) in its 15 days old summer leaves under stressed conditions. However, the monsoon and early winter leaves responded positively and record a significant ($P<0.01$) increase of about 12% and 18% in their carbohydrate contents respectively. This in turn was followed by a severe decline in late winter samples showing a maximum variation of about 25% ($P<0.01$) compared to control. In S. cumini the decrease in carbohydrate contents was observed in the samples of all the seasons. However, the losses were significant ($P<0.01$) only in summer and monsoon samples exhibiting a depletion of 28% and 26% respectively. E. citriodora experienced a heavy loss of about 17% ($P<0.01$) in its carbohydrate contents in the summer foliage, whereas, in the leaves collected in monsoon and late winter, the decrease was non-significant compared to control. On the other hand, the early winter leaves in this species exhibited a considerable increase of 15% ($P<0.05$) in its carbohydrate level

in the population growing at the polluted site. In D. sissoo the carbohydrate levels suffered a marked decline in the 15 days old summer foliage (19%) ($P < 0.01$), whereas, the leaves collected in the subsequent seasons did not differ significantly in their carbohydrate contents in either of the sites. On the other hand, monsoon samples of T. grandis showed a decline of about 14% ($P < 0.05$) in the polluted environment. However, there was a significant ($P < 0.05$) increase of about 11% and 12% in the carbohydrate contents of the foliage collected in early and late winter season. 15 days old summer samples of this species though exhibited higher values in the abnormal atmosphere, the variation compared to control on statistical analysis proved to be non-critical.

The concentration of carbohydrate varied in the leaves of different seasons as well as in various species undertaken for investigation in the present study. The trend in the change of concentration in M. indica and T. grandis followed a similar pattern. In both these species, after an initial increase in carbohydrate concentration, the maximum was recorded in monsoon samples followed by a gradual decline as the season advanced to record the minimum in late winter. Contrary to this, in the other two species P. quajava and E. citriodora there is a gradual increase in carbohydrate concentration upto early winter season where it showed its peak value followed by a fall in the late winter. In the remaining two species, S. cumini and D.

Table-23:- Data on the total carbohydrate contents (percent dry weight) in the foliage of various tree species in different seasons.

Species	Site	Summer	Monsoon	Early Winter	Late Winter
<i>M. indica</i>	P	15.00±1.33	27.00±1.39	25.00±1.29	22.80±1.84
	C	15.40±1.00	29.00±1.61	26.00±1.93	24.40±1.56
		(-3) ^{NS}	(-7) ^{NS}	(-4) ^{NS}	(-7) ^{NS}
<i>P. guajava</i>	P	8.00±0.52	15.20±0.53	20.00±0.70	12.20±0.84
	C	9.80±0.56	13.60±0.45	17.00±0.80	16.20±0.78
		(-18) ^{**}	(+12) ^{**}	(+18) ^{**}	(-25) ^{**}
<i>S. cumini</i>	P	6.20±0.87	13.40±0.59	12.60±1.12	13.20±0.59
	C	8.60±0.45	18.00±0.70	14.00±1.08	14.20±1.14
		(-28) ^{**}	(-26) ^{**}	(-10) ^{NS}	(-7) ^{NS}
<i>E. citriodora</i>	P	7.20±0.52	14.80±0.56	18.40±1.04	14.20±0.52
	C	8.70±0.48	15.20±0.64	16.00±1.36	15.00±0.59
		(-17) ^{**}	(-3) ^{NS}	(+15) [*]	(-5) ^{NS}
<i>D. sissoo</i>	P	12.00±0.54	18.00±0.64	16.40±1.26	17.50±0.96
	C	14.80±0.59	17.60±0.42	16.80±0.54	17.00±0.86
		(-19) ^{**}	(+2) ^{NS}	(-2) ^{NS}	(+3) ^{NS}
<i>L. grandis</i>	P	17.40±0.96	21.00±1.94	19.60±1.32	19.00±1.21
	C	16.60±1.00	24.40±1.66	17.60±1.02	16.90±1.21
		(+5) ^{NS}	(-14) [*]	(+11) [*]	(+12) [*]

Values are mean ± standard deviation

P = Polluted, C = Control

** = Significant at P<0.01 level

* = Significant at P<0.05 level

NS = Non-significant

(+) and (-) indicate percent increase and decrease over control respectively

sissoo the highest carbohydrate contents were observed in the monsoon samples, followed by a fall in early winter which again showed an enhancement in late winter samples. Among the various species the carbohydrate concentration was found maximum in the monsoon samples of M. indica (29%) and the lowest in the 15 days old summer samples of S. cumini (6.20%), the former collected from the control and the latter from the polluted site.

Carbohydrate in Bark :-

The concentration of carbohydrate in the bark samples of various species investigated in the present study (table-24) shows that in M. indica elevated levels of carbohydrate are found in the polluted samples in all seasons. The increase ($P < 0.01$) was the highest in summer (30%), followed by monsoon (24%) and the lowest in winter season (23%). In P. guajava, except in the monsoon season where the difference was only marginal, the summer and winter samples exhibited significantly ($P < 0.01$) higher carbohydrate levels showing a variation of 12% and 14% respectively. On the other hand, E. citriodora recorded significantly ($P < 0.01$) lower levels of carbohydrate in its summer and monsoon samples, showing a loss of 23% in the former and 20% in the latter season. The loss in the winter samples in this species on statistical analysis proved to be non-critical. The other three species namely S. cumini, D. sissoo and I. grandis did not show any marked variations in their carbohydrate

Table-24:- Data on the seasonal changes in the total carbohydrate contents (percent dry weight) in the bark samples of various tree species.

Species	Site	Summer	Monsoon	Winter
<u>M. indica</u>	P	17.10±0.73	13.40±0.73	16.00±0.68
	C	13.20±0.59 (+30)**	10.80±0.80 (+24)**	13.00±0.54 (+23)**
<u>P. guajava</u>	P	14.80±0.59	11.80±0.40	14.60±0.73
	C	13.20±0.70 (+12)**	11.50±0.36 (+3) NS	12.80±0.70 (+14)**
<u>S. cumini</u>	P	13.00±0.54	14.40±0.42	15.60±0.73
	C	13.50±0.34 (-4) NS	15.40±0.45 (-6) NS	15.60±0.40 (00)
<u>E. citriodora</u>	P	9.60±0.70	9.20±0.50	9.00±0.73
	C	12.50±0.88 (-23)**	11.50±1.14 (-20)**	9.50±1.66 (-5) NS
<u>D. sissoo</u>	P	14.40±0.45	14.80±1.03	15.20±0.64
	C	14.40±0.66 (00)	13.80±0.54 (+7) NS	15.20±0.56 (00)
<u>I. grandis</u>	P	12.40±0.45	12.50±0.34	13.40±0.45
	C	11.80±1.16 (+5) NS	12.00±0.54 (+4) NS	13.20±0.40 (+2) NS

Values are mean ± standard deviation
P = Polluted, C = Control
** = Significant at P<0.01 level
NS = Non-significant
(+) and (-) indicate percent increase and decrease over control respectively.

contents in the polluted samples compared to control.

The concentration of carbohydrate though variable in different species, the change followed the same trend in polluted and control samples of a particular species. The concentration of carbohydrate compared to other seasons was maximum in the summer samples of M. indica, P. quajava and E. citriodora, whereas, the other species viz. S. cumini, D. sissoo and I. grandis recorded the highest amount in their winter samples regardless of the site of collection.

Carbohydrate in Wood :-

The data shows that (table-25) carbohydrate contents in the wood samples of various tree species was lower in the population growing at normal site than at the polluted site. While M. indica, P. quajava and I. grandis record a non-significant increase, the rest of the species observed significantly elevated levels of carbohydrate in their polluted samples. In S. cumini the increase was about 35% ($P < 0.01$) in the summer season, whereas, the monsoon and winter samples, show an enhancement of about 17% and 16% ($P < 0.01$) over control respectively. In E. citriodora, significantly ($P < 0.01$) higher amounts of carbohydrate were found only in monsoon and winter seasons showing an increase of 19% and 23% respectively. On the other hand, D. sissoo exhibited a significant ($P < 0.01$) increment

Table-25:- Data on the seasonal alterations in the total carbohydrate contents (percent dry weight) in the wood samples of various tree species.

Species	Site	Summer	Monsoon	Winter
<u>M. indica</u>	P	17.20±1.18	17.60±0.66	17.10±0.87
	C	16.80±0.50	17.00±0.56	16.60±0.70
		(+2) NS	(+4) NS	(+3) NS
<u>P. guajava</u>	P	23.00±0.70	21.60±0.73	20.00±1.78
	C	22.70±1.14	21.00±0.34	18.40±1.03
		(+1) NS	(+3) NS	(+9) NS
<u>S. cumini</u>	P	17.60±0.73	19.20±0.70	18.40±0.42
	C	13.00±0.59	16.40±0.73	15.80±0.56
		(+35) **	(+17) **	(+16) **
<u>E. citriodora</u>	P	20.00±1.66	18.80±0.80	19.60±0.73
	C	18.80±0.56	15.80±0.80	16.00±0.70
		(+6) NS	(+19) **	(+23) **
<u>D. sissoo</u>	P	27.00±0.77	26.40±0.68	26.60±0.49
	C	22.40±0.70	20.00±0.82	22.00±0.45
		(+21) **	(+32) **	(+21) **
<u>T. grandis</u>	P	23.80±2.40	22.40±0.64	22.00±1.12
	C	22.00±1.76	21.50±0.64	21.00±1.14
		(+8) NS	(+4) NS	(+5) NS

Values are mean ± standard deviation

P = Polluted, C = Control

** = Significant at P<0.01 level

NS = Non-significant

(+) indicates percent increase over control.

of about 21%, 32% and 21% in summer, monsoon and winter seasons respectively.

The amount of carbohydrate in different seasons was the maximum in monsoon in case of M. indica and S. cumini, whereas, the rest of the species viz. P. guajava, E. citriodora, D. sissoo and I. grandis recorded the peak concentrations in their summer samples. The concentration of carbohydrate was varied in different species, however, the change of concentration showed similarity in control and polluted samples. Further, the concentration of carbohydrate in different species was found the highest in polluted samples of D. sissoo (27%) and the lowest in control samples of S. cumini (13%), both collected in the summer season.

8.4. D I S C U S S I O N

Carbohydrate in Leaves :-

The studies undertaken on the impact of air pollution on the carbohydrate levels in the plants have shown that SO_2 significantly reduced the contents of carbohydrates in Ulmus americana (Constantinidou and Kozlowski, 1979); Triticum aestivum (Prasad, 1980); Pinus strobus (Percy and Riding, 1981); Glycine max and Triticum aestivum (Prasad and Rao, 1982); Avena sativa (Chand and Kumar, 1987); Syzygium cumini (Vijayan and Bedi, 1988); Lycopersicon esculentum and Hordeum vulgare

(Prakash et al., 1989a, b) and Polyalthia longifolia (Singh, 1989). Similarly starch, sugar and even fructose levels have been found to get decreased due to SO₂ pollution (Devi and Patel, 1983; Keul et al., 1984; L'Hirondelle and Addison, 1985; Mejnartowicz and Lukasiak, 1985; Sharma and Rao, 1985; Krishnamurthy et al., 1986; Nandi et al., 1986). However, in most of the other cases of exposure to SO₂, plants have been reported to exhibit increasing amount of soluble and reducing sugars (Khan and Malhotra, 1977; Koziol and Jordan, 1978; Malhotra and Sarkar, 1979; Agrawal, 1982; Farooq and Beg, 1982; Krishnamurthy et al., 1986; Nandi et al., 1986; Farooq et al., 1988).

The data obtained in the present study on the concentration of carbohydrates in the leaves collected in various seasons from different tree species growing at polluted as well as at normal sites is presented in table (23). The observations indicate that the concentration of carbohydrate was significantly higher in monsoon and early winter leaves of P. guajava at the polluted site, whereas, its summer as well as late winter foliage show a marked decrease in the same environmental set-up. However, M. indica did not show any significant variation in the carbohydrate concentration in its foliage in all the seasons under pollution stress. On the other hand, S. cumini exhibited significantly reduced levels in its summer and monsoon samples, whereas, in E. citriodora and D.

sissoo the marked reductions were observed only in their summer leaves. However, in E. citriodora there was a considerably elevated level of carbohydrate in the early winter season. I. grandis experienced severe losses in monsoon, although, the carbohydrate contents increased to a significant level in the early and late winter seasons. Further, the concentration of carbohydrate though varied in different species in different seasons, the trend of change in the concentration was similar in polluted as well as in control samples. The foliage showed the maximum concentration of carbohydrate in monsoon season in case of M. indica, S. cumini, D. sissoo and I. grandis, whereas, the other two species viz. P. guajava and E. citriodora exhibit the highest concentration in early winter samples. Moreover, universally in all the investigated species, the lowest concentration of carbohydrate was found in summer samples.

The data further shows that among various species growing at the polluted site the maximum reduction of 28% was observed in the 15 days old summer leaves of S. cumini and the minimum loss of (2%) in the early winter sample of D. sissoo. On the other hand, an increase of about 18% was recorded in the early winter leaves of P. guajava and a marginal increase of about 2% in the monsoon samples of D. sissoo. The reductions in the carbohydrate contents as observed in the foliage of various tree species in the present study may be due to the reduced photosynthetic activity because of the reductions in the

amount of photosynthetic pigments due to pollution stress. However, Pierre and Queiroz (1981) held that reductions in carbohydrate levels are independent of chlorophyll loss and further suggested a number of metabolic key enzymes as the primary targets of sub-necrotic SO_2 levels. The losses in the carbohydrate levels in the population growing at the polluted site may also be attributed to the reduced CO_2 fixation (Sij et al., 1973) and/or from increased respiration (Syratt and Wanstall, 1969). On the other hand, the increase in the carbohydrate contents in the leaves in various seasons at the polluted site may be due to the lesser utilization of it due to reduced growth activity of the plants as well as the inhibition of other metabolic processes involved in carbohydrate metabolism. The accumulation of carbohydrate in the foliage to a significant level in the SO_2 enriched atmosphere may also be due to the inhibition in its translocation from the leaves (Noyes, 1980), probably because of the phloem loading due to SO_2 pollution (Koziol, 1984).

Carbohydrate in Bark :-

The concentration of carbohydrate in the bark samples of different tree species and the percent variations between control and polluted samples is given in table (24). The data shows that in M. indica and P. guajava significantly elevated levels of carbohydrate are found in all seasons at the polluted

site except in the monsoon samples of latter where the difference was only marginal. On the other hand, a marked loss in carbohydrate contents was observed in the summer and monsoon samples of E. citriodora in SO₂ enriched environment. Whereas, the other three species viz. S. cumini, D. sissoo and I. grandis did not exhibit any significant changes in their carbohydrate contents under pollution stress. Patel and Devi (1986) also observed reduced levels of starch in the bark and wood samples of S. cumini and M. indica populations growing in the polluted atmosphere around a fertilizer complex. However, an increase in the amount of starch was observed in Tamarindus indica growing at the same site. Further, among the various species investigated in the present study, the lowest concentration of carbohydrate was observed in the polluted samples of E. citriodora (9%) and the maximum in the polluted samples of M. indica (17.10%), the former collected in the winter and the latter in summer season. Though the contents of carbohydrate varied in different seasons, the pattern of change was the same in polluted as well as in control samples. Further, the three species viz. M. indica, P. guajava and E. citriodora showed the maximum concentration of carbohydrate in summer season, while in the others the peak values were recorded in winter samples.

Carbohydrate in Wood :-

The data (table-25) regarding the amount of carbohydrate

contents in the wood samples of the species investigated in the present study reveals that in general all the species recorded higher carbohydrate concentrations in the wood than in bark or leaves, except in M. indica where the concentration was the highest in foliage followed by wood and the least in bark. It seems that carbohydrates does not even get stored in phloem tissues through which it is transported from the leaves and subsequently through ray system passes into xylem. Since wood is the inner most tissue and well protected from the external environment, higher plants particularly tree species tend to store maximum amount of food in the wood tissue.

In comparison to control, the concentration of carbohydrate was higher in wood samples collected from the polluted site in all the investigated species. This indicates lesser utilization of it due to reduced growth activity in the pollution affected population. Moreover, like in bark and leaves the trend of change in carbohydrate concentration was similar in the polluted and control samples in all the species investigated in the present study.

9. COAL SMOKE POLLUTION AND PROTEIN SYNTHESIS

9.1. INTRODUCTION

The proteins are the most vital components of the living systems and their most significant influence resides in the fact that many are functionally active as enzymes which are vital for the rapid rates of biochemical reactions. Proteins also act as natural hydrogen ion buffers and structural components of the cells. The literature pertaining to the impact of SO_2 on the protein contents of the plants is well documented. In a very recent communication Rao and Dubey (1990 a,b) reported significantly reduced protein contents in the foliage of several plant species viz. Azadirachta indica, Calotropis procera, Cassia siamea, Dalbergia sissoo, Ipoemea fistulosa, Mangifera indica, Syzygium cumini and Zizyphus mauritiana growing in field conditions around an industrial area. In an another detailed investigation on certain tropical tree species, Beg and Farooq (1988) observed that SO_2 concentrations which produced no visible injury, the plants like Ficus rumphii, Holoptelea integrifolia, Mangifera indica, Psidium guajava and Syzygium cumini exhibit marked decrease in the protein contents. However, no change was observed in the Pithecolobium dulce while Ficus

berghalensis shows elevated protein contents in its tissues.

9.2. METHODOLOGY

The protein contents in the samples was estimated by following the method of Lowry et al. (1951) (Sadasivum and Manikam, 1992b). One gram of fresh leaf sample (for bark and wood dry samples were used) was homogenised with 5-10 ml of phosphate buffer (pH 7-9) in a glass mortar. The solution was centrifuged and the supernatant used for protein estimation. 0.1 ml and 0.2 ml of the sample extract was pipetted out in two graduated tubes and volume made upto 1 ml in each tube. Another tube with 1 ml of water served as blank. To each of these test tubes was added 5 ml of Reagent C (Appendix-3.3). The solution was mixed well and allowed to stand for 10 minutes. Then 0.5 ml of Reagent D (Appendix-3.4) was added to this solution in each tube and incubated at room temperature in dark for 30 minutes. The blue colour developed was read at 660 nm on a "spectronic-20" colorimeter. The protein content was estimated by comparing the optical density of each sample with a calibration curve plotted by taking known dilutions of a standard solution of bovine serum albumin (Fraction V)

9.3. OBSERVATIONS

Protein in Leaves :-

The data on the seasonal variations in the protein contents

estimated in the foliage of different experimental trees is presented in table (26). The observations clearly indicate that in the population of M. indica growing at the polluted site, there is a marked ($P<0.01$) decrease of about 15% and 22% in the summer and monsoon foliage respectively. However, a tendency towards increased protein levels was observed in early winter samples and the difference was significantly ($P<0.01$) high (51%) in the polluted samples of late winter season. P. guajava too experienced a significant ($P<0.01$) loss of about 19% in the 15 days old summer leaves, whereas, the foliage collected in the monsoon and winter seasons recorded elevated levels of protein in the abnormal atmosphere. However, the variation was only significant in early and late winter seasons showing an increase of about 13% ($P<0.05$) and 25% ($P<0.01$) over control respectively. The behaviour of S. cumini was different in respect to other species. In this species, the foliage of all the seasons exhibited higher protein contents in the population growing in SO_2 enriched atmosphere. The protein contents were significantly ($P<0.01$) higher in all the seasons except in the monsoon, where the difference was non-significant. The highest increase in the protein contents was found in early winter samples (36%) followed by late winter (34%) and the minimum was recorded in the samples collected in summer (18%). In E. citriodora the pollution caused a significant ($P<0.01$) increase of about 29% in the protein contents in the monsoon foliage.

However, the protein contents in the leaves of other seasons did not show any significant response under pollution stress. While in D. sissoo an enhanced amount of protein was observed in the summer (26%) and early winter foliage (16%) ($P < 0.01$), the monsoon samples suffered significantly ($P < 0.01$) higher losses (17%) due to pollution stress. The protein contents also depleted in the polluted samples of I. grandis collected in summer, monsoon and early winter, with significant ($P < 0.01$) reductions recorded only in summer (18%) and early winter season (19%). However, the late winter foliage in this species responded positively, but the percent increase on statistical analysis proved to be non-significant.

The data further shows that the concentration of protein was variable in different species and even in the foliage of different seasons. However, in a particular species the trend of change in the protein contents showed resemblance (table-26). The protein contents in the foliage collected in various seasons showed its maximum concentration in the monsoon samples of M. indica, P. quajava and I. grandis followed by a continuous fall as the season proceeded to record its minimum value in the late winter season. The other two species S. cumini and D. sissoo showed the maximum concentration in their early winter samples. On the other hand, E. citriodora responded differently exhibiting the peak concentration in its 15 days old summer foliage followed by a steady decline to show the lowest

Table-26:- Data on the protein contents (mg/g fresh weight) in the foliage of various tree species in different seasons.

Species	Site	Summer	Monsoon	Early Winter	Late Winter
<u>M. indica</u>	P	11.65±1.04	17.95±1.20	13.95±0.68	7.70±0.57
	C	13.65±0.61 (-15)**	22.95±1.48 (-22)**	13.10±0.64 (+6) ^{NS}	5.10±0.57 (+51)**
<u>P. guajava</u>	P	13.10±0.80	17.95±0.62	15.40±0.80	7.10±0.39
	C	16.25±0.73 (-19)**	16.80±1.09 (+7) ^{NS}	13.65±0.74 (+13)*	5.70±0.39 (+25)**
<u>S. cumini</u>	P	9.10±0.80	5.98±1.06	11.65±0.74	8.80±0.87
	C	7.70±0.39 (+18)**	5.70±0.45 (+5) ^{NS}	8.55±0.96 (+36)**	6.55±0.74 (+34)**
<u>E. citriodora</u>	P	21.95±1.04	19.10±1.15	13.65±0.80	7.95±0.74
	C	21.35±0.96 (+3) ^{NS}	14.80±0.80 (+29)**	14.25±0.64 (-4) ^{NS}	8.25±0.39 (-4) ^{NS}
<u>D. sissoo</u>	P	13.95±0.90	15.40±0.80	22.50±0.96	14.25±0.39
	C	11.10±0.74 (+26)**	18.50±0.80 (-17)**	19.35±0.57 (+16)**	13.40±0.80 (+6) ^{NS}
<u>I. grandis</u>	P	10.55±0.64	20.80±0.97	17.35±0.97	9.10±0.64
	C	12.80±1.14 (-18)**	22.20±0.80 (-6) ^{NS}	21.35±0.74 (-19)**	8.55±1.04 (+6) ^{NS}

Values are mean ± standard deviation

P = Polluted, C = Control

** = Significant at P<0.01 level

* = Significant at P<0.05 level

NS = Non-significant

(+) and (-) indicate percent increase and decrease over control respectively.

concentration in the late winter season. Further, among the various species investigated in the present study, the maximum protein content was observed in the early winter samples of D. sissoo (22.50%) and the minimum in the late winter foliage of M. indica (5.10%), the former collected from the polluted and the latter from the control site.

Protein in Bark :-

The data on the contents of proteins in the bark samples collected in different seasons from the species growing at the test site as well as control site is summarized in table (27). The data reveals that the protein contents increased to a significant level of 30% ($P<0.05$) and 52% ($P<0.01$) in the summer samples of M. indica and S. cumini respectively both collected from the polluted site. However, no marked variation was observed in their monsoon and winter samples. On the other hand, D. sissoo exhibited heavy losses ($P<0.01$) in its protein contents in the summer and monsoon seasons, the decrease being slightly higher in the former (24%) than in the latter season (21%). The other species like P. guajava, E. citriodora and I. grandis although exhibited apparently high values of protein contents in their bark samples at the polluted site, the difference compared to control on statistical analysis proved to be non-significant.

Table-27:- Seasonal changes in the protein contents (mg/g dry weight) in the bark samples of various tree species.

Species	Site	Summer	Monsoon	Winter
<u>M. indica</u>	P	2.45±0.376	3.82±0.438	2.50±0.262
	C	1.88±0.359 (+30)*	3.30±0.319 (+15) ^{NS}	2.28±0.387 (+10) ^{NS}
<u>P. quajava</u>	P	1.88±0.239	3.14±0.182	5.36±0.387
	C	1.70±0.114 (+11) ^{NS}	2.85±0.399 (+10) ^{NS}	4.90±0.479 (+9) ^{NS}
<u>S. cumini</u>	P	2.85±0.399	4.27±0.324	5.47±0.450
	C	1.88±0.365 (+52)**	3.99±0.239 (+7) ^{NS}	5.00±0.364 (+9) ^{NS}
<u>E. citriodora</u>	P	2.85±0.399	5.64±0.535	4.56±0.239
	C	2.85±0.176 (00)	5.13±0.604 (+10) ^{NS}	4.21±0.256 (+8) ^{NS}
<u>D. sissoo</u>	P	3.70±0.205	3.99±0.193	4.56±0.416
	C	4.84±0.228 (-24)**	5.07±0.279 (-21)**	5.13±0.387 (-11) ^{NS}
<u>I. grandis</u>	P	3.53±0.256	5.41±0.399	4.27±0.387
	C	3.42±0.148 (+3) ^{NS}	5.01±0.285 (+8) ^{NS}	3.99±0.193 (+7) ^{NS}

Values are mean ± standard deviation

P = Polluted, C = Control

** = Significant at P<0.01 level

* = Significant at P<0.05 level

NS = Non-significant

(+) and (-) indicate percent increase and decrease over control respectively.

Although the amount of protein was variable in different species in different seasons, the change of concentration in the polluted and control samples of a particular species followed the same trend (table-27). Out of six species investigated, three species viz. M. indica, E. citriodora and I. grandis recorded the maximum concentration of proteins in their monsoon samples regardless of the site of collection. Whereas, the other ones viz. P. guajava, S. cumini and D. sissoo observed the highest concentration in their winter samples.

Protein in Wood :-

The observations recorded (table-28) on the seasonal variations in the protein contents in various species under investigation show that highly significant ($P < 0.01$) losses were observed in all the seasons in case of M. indica population growing at the polluted site. The percent loss was greater in the summer season (27%) and less in the monsoon season (20%). E. citriodora also responded negatively in the polluted environment experiencing severe losses of 33% and 21% ($P < 0.01$) in its summer and monsoon samples respectively, whereas, D. sissoo recorded marked reductions only in its winter samples (23%) ($P < 0.01$). In contrast, a highly significant ($P < 0.01$) increase in protein contents was observed in the monsoon and winter samples of S. cumini showing a variation of 33% and 56% respectively. I.

Table-28:- Seasonal alterations in the protein contents (mg/g dry weight) in the wood samples of various tree species.

Species	Site	Summer	Monsoon	Winter
<u>M. indica</u>	P	2.50±0.205	7.58±0.273	5.47±0.410
	C	3.42±0.199 (-27)**	9.46±0.615 (-20)**	7.00±0.490 (-22)**
<u>P. guajava</u>	P	2.45±0.273	3.59±0.324	6.15±0.929
	C	2.45±0.359 (00)	3.93±0.307 (-9)NS	6.95±0.615 (-11)NS
<u>S. cumini</u>	P	3.82±0.262	5.70±0.438	8.55±0.763
	C	3.42±0.399 (+12)NS	4.27±0.182 (+33)**	5.47±0.416 (+56)**
<u>E. citriodora</u>	P	2.85±0.366	7.18±0.416	4.04±0.370
	C	4.27±0.228 (-33)**	9.12±0.399 (-21)**	4.10±0.324 (-1)NS
<u>D. sissoo</u>	P	4.90±0.364	5.01±0.376	6.27±0.456
	C	4.95±0.273 (-1)NS	5.70±0.592 (-12)NS	8.15±0.478 (-23)**
<u>I. grandis</u>	P	5.30±0.364	5.70±0.433	8.03±0.399
	C	3.70±0.205 (+43)**	5.70±0.399 (00)	6.61±0.256 (+21)**

Values are mean ± standard deviation

P = Polluted, C = Control

** = Significant at P<0.01 level

NS = Non-significant

(+) and (-) indicate percent increase and decrease over control respectively.

grandis also exhibited significantly elevated level of protein contents in its summer (43%) and winter samples (21%) at (P<0.01) level, whereas, its monsoon samples showed equal amounts in polluted as well as in the control site. The data also indicates that among the different seasons, the concentration of protein was maximum in the monsoon samples in case of M. indica and E. citriodora, while, the rest of the species exhibit the peak concentrations in their winter samples. Moreover, the concentration of protein though variable among different species, the change of concentration in a particular species followed the similar pattern in polluted as well as control samples (table-28).

9.4. D I S C U S S I O N

Protein in Leaves :-

The data (table-26) pertaining to the contents of proteins in the foliage of different tree species undertaken for investigation in the present study shows that coal smoke pollution reduced the amount of proteins to a highly significant level in the monsoon samples of M. indica showing a loss of about 22%, the highest percent reduction recorded compared to other species. On the other hand, the maximum increase of about 51% in the protein contents was also observed in the leaves of same species in the late winter season under the same

environmental conditions. Similarly in the monsoon samples of E. citriodora a significantly higher level of protein was recorded in polluted samples. While in S. cumini the protein contents increased significantly in the foliage of all seasons although marginally in monsoon samples, in I. grandis except in the leaves of monsoon and late winter seasons, the samples collected in the other seasons experienced severe losses under pollution stress. The other two species viz. P. guajava and D. sissoo recorded losses as well as increased amounts in SO₂ enriched atmosphere depending upon the season of collection. Several workers in the past have reported reduced protein contents in the plants under the stress of SO₂ pollution (Fischer, 1971; Godzik and Linskens, 1974; Mudd, 1975; Constantinidou and Kozlowski, 1979; Malhotra and Sarkar, 1979; Grill et al. 1980; Prasad, 1980; Robe and Kreeb, 1980; Percy and Riding, 1981; Agrawal, 1982; Prasad and Rao, 1982; Singh et al. 1985; Krishnamurthy et al. 1986; Vijayan and Bedi, 1988; Rao and Dubey, 1990 a,b). However, Murray (1985) reported no impact of SO₂ in the leaf protein content of Medicago sativa.

The data (table-26) on the contents of protein in the foliage of various seasons showed its maximum amount in monsoon season for M. indica, P. guajava and I. grandis, while, in S. cumini and D. sissoo the maximum concentration was observed in their early winter samples regardless of the site of collection. E. citriodora was the only species to record maximum

concentration in its 15 days old summer leaves. Moreover, the trend in the change of concentration of proteins followed the same pattern in both the sets of samples (polluted and normal). Interestingly in all the three fruit trees viz. M. indica, P. guajava and S. cumini the protein concentration increased to a significant level in late winter samples, whereas, the other species did not exhibit any marked difference in the foliage of the same season at the test site.

Foliar protein concentrations have previously been shown to record an increase by SO_2 application (Godzik and Linskens, 1974; Prasad and Rao, 1982; Saxe, 1983; Murray, 1984), but the effect is concentration dependent, as exposure to $150 \mu\text{gm}^{-3}$ of SO_2 increased protein concentration in soybeans and peas by stimulating the synthesis of sulphur containing amino acids, but exposure to 500 or $1000 \mu\text{gm}^{-3}$ had inhibitory effects (Sardi, 1981). In an another experiment Jager et al. (1985), exposed sensitive and/resistant pea cultivars to SO_2 and found that protein was increased only in sensitive cultivars as a result of gaseous exposure. The increase in the protein contents in the foliage of different seasons in the species under study may also reflect its enhanced synthesis as the plant attempts to overcome pollutant injury (Craker and Starbuck, 1972).

On the other hand, the decrease in the accumulation of proteins in the polluted samples may be due to the changes in amino acid concentration in SO_2 treated plants which ultimately

lead to the protein reduction (Godzik and Linskens, 1974). Such reductions may be due to the inactivation of enzymes responsible for protein synthesis (Cecil and Wake, 1962). However, Malhotra and Khan, (1984) held that a decrease in the protein contents could be attributed to the breakdown of the existing proteins and to reduced de novo synthesis. Further, it is also possible that the higher concentrations of SO₂ may breakdown enzymes and proteins with disulphide bonds into thio sulphonates and thiols (McMullen, 1960; Loughman, 1964) and thereby hinder the synthesis of proteins. The reduction in the protein contents of SO₂-treated plants might also result from decreased photosynthesis (Sij and Swanson, 1974; Constantinidou and Kozlowski, 1979).

Protein in Bark :-

The observations (table-27) recorded on the contents of proteins in the bark samples of various tree species show that except D. sissoo all other species recorded elevated levels of protein in their polluted samples. However, the significant increase was observed only in the summer samples of M. indica and S. cumini. D. sissoo on the other hand stood alone of its type to exhibit lower protein levels in the SO₂ enriched atmosphere. Further, the concentration of protein was highest in the monsoon samples of M. indica, E. citriodora and I. grandis, whereas, the rest of the species viz. P. guajava, S. cumini and D. sissoo observed

the maximum amount of protein in their winter samples. Moreover, the change in the concentration of protein in the polluted and control samples of a particular species followed a similar pattern.

Protein in Wood :-

The data (table-28) based on the amount of protein in the wood samples of the species under study in the present investigation indicates that highly significant losses in protein contents are found in the polluted samples of M. indica and E. citriodora except in the winter samples of latter in which the difference was only marginal. D. sissoo also experienced severe losses in its protein contents in the winter season. On the other hand, significantly higher amount of protein was observed in the monsoon and winter samples of S. cumini as well as in the summer and winter samples of I. grandis collected from the test site. The trend of change in concentration was similar in both control and polluted samples of all the investigated species. Further, M. indica and E. citriodora exhibited the maximum concentration of protein in their monsoon samples, whereas, the other species viz. P. quajava, S. cumini, D. sissoo and I. grandis recorded the higher values in their winter samples. The data also shows that in general the concentration of protein was observed higher in the wood than in bark samples in all the species investigated in the present study.

10. C O N C L U S I O N

The air pollution caused by coal burning in the thermal power plant complex of Kasimpur causes a serious damage to vegetation in its surrounding areas. The present investigation undertaken to study the effect of air pollution on the biochemical responses of some of the important tree species viz. M. indica, P. guajava, S. cumini, E. citriodora, D. sissoo and I. grandis infers the following.

1. The coal smoke pollution causes an increase in the sulphur content of foliage and barks of all the species irrespective of any seasonal effect.
2. Pollution also affects the nitrogen assimilation in the foliage of all the species, the magnitude of loss or gain depends upon the season as well as the genetic constitution of the species concerned.
3. The phosphorus concentration also exhibits altered rhythms in the foliage of various species under pollution stress. The leaves in various seasons show increased as well as decreased amounts depending on the growth performance of the species concerned.
4. Pollution also causes a serious depletion of K in the foliage, bark and wood of all the investigated species

except *I. grandis* which records elevated levels.

5. The coal smoke pollution considerably affects the chlorophyll pigments as well as carotenoids invariably in all the species, the loss being greater in chlorophyll a than in chlorophyll b.
6. Carbohydrate metabolism does not show any specific trend under pollution stress.
7. Pollution also interferes with the protein synthesis of the plants and the loss or gain depending upon the season as well as the inherent characters of the species concerned.

11. CONCLUDING REMARKS AND RECOMMENDATIONS

Based on the results obtained in the present investigation on the biochemical responses of six selected tree species to coal smoke pollution it may be concluded in general that:

1. The different species respond differently to coal smoke pollution depending on its own genetic constitution and in the climatic condition as well as the part or organ of the concerned plant and, therefore, it becomes inevitable for the investigator to study the individual species as well as the different parts of an individual plant before anything could be said definite about the behaviour of the species as a whole.
2. In general, in a sulphur enriched atmosphere all plants accumulate inorganic sulphur to a highly significant level and, therefore, a green cover of the area around a pollution source like thermal power plants will help to reduce the pollution load of the ambient atmosphere.
3. Increased levels of the organic nitrogen in the growing population of trees around the pollution source play important role being beneficial to soil fertility by the falling plant parts and the decomposing debris.

4. Low level of phosphorus in pollution affected plants is an indication of high energy expenditure by the affected plants in facing the pollution hazard and the possibility of reducing the stress by the manipulation of phosphorus management.
5. The depletion of potassium level in the different species under pollution stress indicates its active role in the ion exchange activity of the plants under stress and thus gives a clear clue for further investigation in this line of study to determine the positive role of potassium in combating pollution hazard by green vegetation.

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A P P E N D I X

The reagents for various biochemical determinations were prepared according to the following procedure.

1. Reagents for Nitrogen estimation

1.1 Nessler's Reagent:-

10 g of potassium iodide was dissolved in 10 ml of distilled water. To this was added a solution of mercuric chloride (6 g in 100 ml of water) in small lots and with shaking till a slight permanent precipitate was formed. To this was added 80 ml of 9 N potassium hydroxide solution and then diluted to 200 ml with distilled water. The solution was kept overnight and the clear solution decanted for use.

2. Reagents for Phosphorus estimation

2.1. Molybdate Reagent:-

6.25 g of ammonium molybdate was dissolved in 75 ml of 10N H_2SO_4 . To this solution 175 ml of distilled water was added in order to get 250 ml of the above reagent.

2.2. Amino naphthol sulphonic acid:-

0.5 g of 1, 2, 4, Amino-naphthol-sulphonic acid was dissolved in 195 ml of 15% sodium bisulphite solution to which 5 ml of 20% sodium sulphite solution was added. The above solution was stored in a dark coloured bottle.

3. Reagents for Protein estimation

3.1. Reagent A:-

2% Sodium carbonate was mixed with 0.1N sodium hydroxide solution.

3.2. Reagent B:-

0.5% Copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) was added to 1% potassium sodium tartrate.

3.3. Reagent C (Alkaline Copper sulphate solution):-

It was prepared by mixing 50 ml of reagent 'A' and 1 ml of reagent 'B' prior to use.

3.4. Reagent D (Folin-Ciocalteu reagent):-

100 g sodium tungstate ($\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$) and 25 g sodium molybdate ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$) was dissolved in 700 ml distilled water in which 50 ml of 85% phosphoric acid and 100 ml of concentrated hydrochloric acid was mixed. The flask was connected with a reflux condenser and boiled gently on a heating mantle for 10 h. At the end of the boiling period, 150 g lithium sulphate, 50 ml distilled water and 3-4 drops of liquid bromine was added to this flask. The reflux condenser was removed and the solution in the flask was boiled for 15 minutes in order to remove excess bromine, cooled and diluted to 1 liter. The strength of this acidic solution (1 N) was tested by treating it with 1 N sodium hydroxide using phenolphthalein as an indicator.